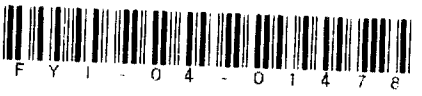
FYI-0804-01478 MR# 278447

DOW CORNING



August 16, 2004

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U.S. Environmental Protection Agency
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Washington, DC 20460

Attn: TSCA Docket Clerk

Re: For Your Information Submission:

The enclosed information is submitted on behalf of Dow Corning Corporation, Midland, Michigan, 48686-0994, on a For-Your-Information (FYI) basis as a follow-up to submissions made concerning dodecamethylcyclohexasiloxane (DDMCHS), which chemical substance was the subject of a health and safety data rule issued under Section 8(d) of the Toxic Substances Control Act (TSCA) and with an effective date of June 14, 1993 (sunset date June 30, 1998), as codified at 40 CFR 716 (Health and Safety Data Reporting). The information presented in this submission was generated as part of our Siloxane Research Program. This program was the subject of a memorandum of understanding, dated April 9, 1996, between Dow Corning and EPA.

Listed Chemical Substance:

540-97-6 Dodecamethylcyclohexasiloxane (DDMCHS, D_6)

Final Study Report:

Disposition of ^{14}C- Dodecamethylcyclohexasiloxane (D_6) following Single, Oral Administration to Fischer 344 Rats

Dow Corning Corporation
2004-I0000-53503
April 28, 2004

Dow Corning Corporation
Midland, Michigan 48686-0994
Phone: (989) 496-4000
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Manufacturer:

Dow Corning Corporation
PO Box 994
2200 West Salzburg Road
Midland, Michigan 48686-0994

For purposes of this TSCA For-Your-Information (FYI) submission, the general INTERNAL designation on the attached health and safety report is waived by Dow Corning.

If you require further information regarding this submission, please contact Michael Thelen, Manager of U.S. EPA Regulatory Affairs, at 989-496-4168 or at the address provided herein.

Sincerely,

Kathleen P. Plotzke

Kathleen P. Plotzke
Director, Health and Environmental Sciences
(989) 496-8046

DOW CORNING CORPORATION
HEALTH & ENVIRONMENTAL SCIENCES
TECHNICAL REPORT

Report No.:	2004-I0000-53503
Title:	Disposition of ^{14}C- Dodecamethylcyclohexasiloxane (D_6) following Single, Oral Administration to Fischer 344 Rats
Study No.:	9683
Test Article:	^{14}C-Dodecamethylcyclohexasiloxane (^{14}C- D_6)
Study Director:	Marina L. Jovanovic, M.S. Associate Toxicology Specialist
Sponsor:	Dow Corning Corporation
HES Management:	Steven D. Crofoot, M.S. Team Leader, Toxicology Health and Environmental Sciences
Testing Facility:	Dow Corning Corporation Health and Environmental Sciences Auburn, Michigan 48611
Study Completion Date:	April 28, 2004
Security Statement:	**Dow Corning Internal.** This report may be reproduced and shared with any Dow Corning employee. Distribution outside the Corporation must be approved by the Director of Health and Environmental Sciences. When this INTERNAL report is no longer needed, it may be placed in office waste baskets for destruction.

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ABSTRACT

The disposition of ^{14}C-dodecamethylcyclohexasiloxane (^{14}C-D_6) was evaluated in male and female Fischer 344 rats following a single, oral administration of 1000 mg of ^{14}C-D_6 in corn oil/kg of body weight. Animals (N=4/sex) were housed in glass metabolism cages for collection of urine, feces and expired air. At 168 hr post-dose, animals were sacrificed and selected tissues and remaining carcasses were collected. All samples were analyzed for radioactivity content. In addition to radioactivity, feces and expired volatiles were analyzed for parent D_6 concentration. A separate group of animals, cannulated *via* jugular vein (N=6/sex), were used to determine radioactivity and parent D_6 concentration in blood at 15 min, 1, 6, 12, 18, 24, 48, 72, 96, 120, 144, 168 hr post dosing. Selected urine and feces samples were analyzed by high performance liquid chromatography with radiochemical detection (HPLC/RAD) in order to evaluate the metabolite profile. Whole-body autoradiography (WBA) was used for qualitative *in vivo* assessment of tissue distribution of radioactivity in male and female rats following a single oral administration of D_6 in corn oil. Animals in the WBA groups were sacrificed at 1, 4, 12, 24, 48, 96, 168 hr post-dose.

The majority of administered dose, regardless of sex, was excreted in feces. The absorption of D_6 based on radioactivity recovered in urine, expired volatiles, expired CO_2, tissues and carcass in males and females dosed with ^{14}C-D_6 in corn oil was 11.88 and 11.83% of administered dose, respectively. Both sexes showed a similar pattern of disposition (Urine: 0.38 and 0.32%; Expired volatiles: 11.20 and 11.21%; Expired CO_2: 0.13 and 0.09%; Tissues: 0.03 and 0.04%; Carcass: 0.14 and 0.17% for males and females, respectively). However, considerable variability was seen in radioactivity levels in expired volatiles (from 3.86 % to 25.28% of administered dose) maybe due to off gassing from the fecal pellets that were not collected as intended but remained inside the cage. This phenomenon could potentially give some false high values for expired volatiles and absorption due to partitioning from the fecal matter into the air. The entire radioactivity in the expired volatiles was attributed to parent D_6. Metabolic profile evaluation of urine and feces showed that the entire radioactivity in the urine consisted of polar metabolites, whereas in the feces the majority was parent D_6 with a trace non-polar metabolite.

Whole body autoradiography data supported mass balance data showing that the majority of administered D_6 in corn oil stayed in the GI tract and was excreted in feces within 48 hours. Low levels of radioactivity were detected in organs and tissues such as liver, fat and bone marrow indicating some absorption of D_6. Statistical analysis of blood curves indicated the presence of small amount of metabolites in the blood based on difference between radioactivity and parent area under the curves
($AUC_{metabolites} = AUC_{radioactivity} - AUC_{parent}$).

GLP COMPLIANCE STATEMENT

The study was conducted in compliance with Environmental Protection Agency Toxic Substances Control Act Good Laboratory Practice Standards 40 CFR Part 792, with the exception of the use of software SAS®, v.8.2 that was not validated. Deviations to the Protocol are listed on the page 27 in the Experimental Design section of this report. There were no circumstances that would negatively impact or bias the results of this study.

Marina Jovanovic — April 28, 2004

Marina L. Jovanovic, M.S.
Associate Toxicology Specialist
Study Director

Date

Steven D. Crofoot — 26 APR 04

Steven D. Crofoot, M.S.
Team Leader, Toxicology
Health and Environmental Sciences

Date

Title: Disposition of ^{14}C-Dodecamethylcyclohexasiloxane (D_6) following Single, Oral Administration to Fischer 344 Rats

Study Number: 9683

This study has been audited by the Dow Corning Corporation Health and Environmental Sciences Quality Assurance Unit according to approved Standard Operating Procedures to assure that the raw data are accurately reflected within this final report. The following are the inspection dates and the dates inspection findings were reported.

Dates of Inspection	Phase Inspected	Findings Reported to Study Director	Findings Reported to Management
15-17 May 02	Draft Protocol Review	17 May 02	22 May 02
12 Jun 02	Dose Solution Preparation	12 Jun 02	14 Jun 02
13 Jun 02	Dosing	13 Jun 02	14 Jun 02
18 Jul 02	Determination of Parent D_6 in Feces	18 Jul 02	26 Jul 02
19-26 Nov 03	Draft Final Report with Test Article Information, Dosing and Animal Records Data	26 Nov 03	19 Dec 03
08-15 Dec 03	Draft Final Report Appendix D, (WBA) and Associated Raw Data	16 Dec 03	23 Jan 04
02-22 Dec 03	Appendix A of the Draft Final Report with Radioactivity Data	22 Dec 03	19 Jan 04
23, 29-30 Dec 03	Appendix C of the Draft Final Report with Statistics Data	30 Dec 03	19 Jan 04
31 Dec 03 and 02-07 Jan 04	Bioanalytical Data and Draft Final Report	07 Jan 04	20 Jan 04
12-17 Jan 04	Final Draft Report	26 Jan 04	10 Feb 04
16-19 Apr 04	Wet Specimen Verification	19 Apr 04	21 Apr 04

Joyce L. Henry
Manager, Quality Assurance
Dow Corning Corporation
Health & Environmental Sciences

22 Apr 04
Date

APPROVAL SIGNATURES

This report consists of pages 1 through 149 including Tables 1 through 13, Figures 1 through 10 and Appendices A through D.

Marina Jovanovic — April 28, 2004

Marina L. Jovanovic, M.S.
Associate Toxicology Specialist
Study Director

Date

Jeremy Durham — 27 April 2004

Jeremy Durham, B.S.
Contributing Scientist, Bioanalytical support
Health and Environmental Sciences

Date

Steven D Crofoot — 26 APR 2004

Steven D. Crofoot, M.S.
Team Leader, Toxicology
Health and Environmental Sciences

Date

STUDY INFORMATION

Study Initiation Date:	05/30/2002
Experimental Start Date:	06/13/2002
Experimental Termination Date:	11/22/2002
Study Completion Date:	04/28/2004
Study Director:	Marina L. Jovanovic, M.S. Associate Toxicology Specialist
Sponsor:	Dow Corning Corporation 2200 W. Salzburg Rd. Auburn, MI 48611
Management:	Steven D. Crofoot Team Leader, Toxicology Health and Environmental Sciences Roy A. Campbell Team Leader, Analytical Chemistry Health and Environmental Sciences
Key Study Personnel:	James W. Crissman, D.V.M., Ph.D., D.A.C.V.P. Joan McMahon, Study Coordinator Jeremy Durham, Contributing Scientist (Bioanalytical support) Debra McNett, Analytical support Jane M. Regan, H.T./M.L.T. (A.S.C.P.) Contributing scientist (Whole Body Autoradiography) Trevor Newhook, Biostatistician Joseph M. Tobin, Supervisor

OBJECTIVE

The objective of this study was to determine the absorption and excretory pathways of the test article, ^{14}C-dodecamethylcyclohexasiloxane (^{14}C-D_6), following oral administration in corn oil.

INTRODUCTION

Oral ingestion in humans may be a route of exposure to ^{14}C-D_6. This study was conducted to provide absorption, distribution, metabolism and elimination (ADME) data that may be useful in understanding this route of exposure. Oral gavage is a common and an accepted method of administration of test chemicals in pharmacokinetic and metabolism studies. The experimental design of this study followed test guidelines and Tier 1 data requirements for the health effects testing of pesticides and toxic substances issued in August 1998 by the United States Environmental Protection Agency (EPA). Animals were dosed with ^{14}C-D_6 suspended in corn oil. Corn oil is a commonly used carrier for hydrophobic materials that are administered by oral gavage.

Three experimental approaches were used to provide ADME data:
1) Animals in mass balance groups (MB) were housed in glass metabolism cages for quantitative assessment of D_6 excretion and absorption after oral administration; 2) A group of rats cannulated *via* the jugular vein were used to obtain estimates of blood concentration and basic pharmacokinetic parameters (e.g. area under the curve (AUC) and half-life ($T_{1/2}$)) and 3) Whole-body autoradiography was used for qualitative *in vivo* assessment of tissue distribution of radiolabeled D_6 and its potential metabolites, and for tracking the transient time of radioactivity through the gastrointestinal tract.

TEST SYSTEM

A. Species:	*Rattus norvegicus*
B. Strain/substrain:	CDF® (Fischer 344)/CrlBR
C. Source:	The Charles River Laboratories, Inc. Raleigh, NC
D. Number of groups:	10
E. Number of Animals:	42

(D_6 in Corn Oil):

Mass balance (MB) groups:	Group 1 (MB controls):	2 females
	Group 2 (MB controls):	2 males
	Group 3 (MB):	4 females
	Group 4 (MB):	4 males
Whole body autoradiography: (WBA)	Group 5 (WBA):	7 females
	Group 6 (WBA):	7 males
Blood kinetics (BK):	Group 7 (BK controls):	2 females (C)
	Group 8 (BK controls):	2 males (C)
	Group 9 (BK):	6 females (C)
	Group 10 (BK):	6 males (C)

C = rats cannulated *via* jugular vein

F. Total number: Non-cannulated (NC): 26 (13 females and 13 males)
Cannulated (C): 16 (8 females and 8 males)

G. Special Consideration: Animals in groups 7, 8, 9 and 10 were jugular vein cannulated by the supplier prior to arrival at the testing facility. Extra rats were received for randomization and substitution.

I. Body Weight Range: Females: 133-155g on day of dosing
Males: 163-219g on day of dosing

J. Approximate Age: 8-10 weeks on day of dosing

K. Quarantine: 1 day for cannulated rats
7 days for non-cannulated rats

L. Identification Method:

Upon receipt in the Toxicology Department, each animal received a Q number. At the end of the quarantine period, non-cannulated and cannulated rats were weighed, randomized and uniquely identified by a metal ear-tag displaying the animal number as documented in the study records. Cannulated rats were also checked for cannula patency after they were released from quarantine and prior to random assignment to control and exposure groups. Individual cage tags were placed on the outside of each cage.

M. Method of Euthanasia:

1. **Scheduled animal termination.**

   Immediately prior to euthanasia animals in the Mass Balance (MB) groups were anesthetized using a 15% isoflurane in mineral oil emulsion. All animals in the MB groups were euthanized by exsanguination *via* cardiac puncture under isoflurane anesthesia. Animals in the WBA groups were euthanized by CO_2 asphyxiation. Cannulated rats were euthanized by CO_2 asphyxiation after the last blood collection. If the last blood collection *via* the jugular cannula was not successful, blood was collected by an open thoracic cardiac puncture under isoflurane anesthesia.

2. **Unscheduled death and termination**. A female rat No. D0161 in the blood kinetics group died during the blood collection at the 48 hr timepoint. Female rat No. D0164 was sacrificed at the 144 hr time point due to distress caused by difficulties from bleeding through the cannula. In order to obtain a blood sample, the animal No. D0164 was euthanized by cardiac puncture under isoflurane anesthesia.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

This species and strain of animal is recognized as appropriate for toxicity studies. Fischer 344 female and male rats have previously been used in pharmacokinetic and metabolism studies of various silicone materials, and data obtained in this study can be used as historical data. The use of both sexes enabled detection of any potential gender-related differences in absorption of D_6 from the gastrointestinal (GI) tract. The number of animals used in the mass balance groups was selected to provide statistical power (N=4). Based on previous work with octamethylcyclotetrasiloxane (D_4) and decamethylcyclopentsiloxane (D_5) (Study No. 8546 and Study No. 9550, respectively), the minimum number of time points were chosen for WBA to allow visual assessment of the *in vivo* distribution pattern of radioactivity, as well as track the transient time of radioactivity in the gastrointestinal tract. Also, blood collection time points in the blood kinetics group were selected based on previous work with D_4 and D_5.

METHOD OF RANDOMIZATION

Upon release from quarantine, non-cannulated rats were weighed, and then randomized by weight stratification into test groups using a table of random numbers generated by MicroSoft™ Excel 2000. Cannulated rats were checked for cannula patency prior to randomization. All the animals had patent cannulae. Extra cannulated rats, females No. D0175 and D0176, and males No. D0177 and D0178, were treated as spares. This was considered necessary because of difficulties that are known to occur when

jugular vein cannulated rats are used. Also one extra female rat, No. D0173 and one extra male rat, No. D0174 were treated as spares in MB and WBA groups because of possible difficulties during dosing *via* oral gavage. All animals were within ± 20 % of the mean body weight for the group to which they were assigned. Animals not used on the study were returned to the Animal Resources Group.

SPECIFIC HOUSING AND MAINTENANCE

A. Animal Receipt and Quarantine

All animals received from Charles River Laboratories were judged to be in good health and suitable as test animals. The attending veterinarian examined all animals before release from quarantine and this is documented in the study records.

B. Animal housing

Animals were individually housed in suspended wire-mesh cages (7"x10"x7") during quarantine. The cages were elevated above Bed-O'Cobs® bedding, and were subjected to routine cleaning. Upon release from quarantine rats were individually housed in suspended wire-mesh cages elevated above Bed-O'Cobs®. Animals that were used in MB groups were transferred to individual Roth-style glass metabolism cages and allowed to acclimate overnight to this experimental environment prior to administration of test article. Animals that were used in BK and WBA groups continued to be housed individually in suspended wire-mesh cages throughout the conduct of the study.

All animals were housed in an environmentally controlled animal room (12-hour fluorescent-light/dark cycle, 64-79°F, 30-70% humidity, 10-15 air changes per hour) through the in-life part of the study. Temperature and humidity were monitored twice a day on weekdays and once a day during weekends. The light cycle was only interrupted periodically for sample collection. Such interruptions were necessary for the conduct of the study, and are not considered to have had an impact on the study outcome. Airflow and animal condition in the metabolism cages were monitored at least twice a day (am/pm) during the experiment. Airflow in the metabolism cages was kept in the range of 590-700 ml/min. Cage temperature was checked at the time of sample collection and was kept in the range of 66-70°F.

A commercial diet of Purina® Certified Rodent Chow #5002 (Lot number JAN 2402, 3A) and reverse osmosis (RO) water (Edstrom Industries, Inc. Waterford, WI) were available *ad libitum*. Periodic analysis of the certified feed for the presence of heavy metals and pesticides was performed and provided by the manufacturer to ensure that none were present in concentrations that would be expected

to affect the outcome of the study. Results of the most recent water analysis, provided by an independent laboratory (Ann Arbor Technical Services, Ann Arbor, MI), and feed analysis were reviewed by the study director. Documentation of study director reviews was placed in the study records. There were no contaminants in the water or feed identified at levels that would interfere with the integrity of the study.

ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the final rules of the animal Welfare Act regulations (9 CFR, Part 1, 2 and 3) and was approved by the Laboratory Animal Care and Use Committee (LACUC).

TEST ARTICLE INFORMATION

UNLABELED TEST ARTICLE

Test article characterization was done in compliance with the EPA Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards (40 CFR Part 792).

The characterization of the unlabeled test article (D_6) identified below included a visual inspection, purity by gas chromatography (GC) with thermal conductivity detector (TCD) and GC with mass spectrometry (MS) to verify the identity of the major component as D_6 (HES study No. 8825). Records of characterization are maintained in the HES Archive. Documentation of study director review is kept in the study files. Any remaining test article was disposed by the study personnel.

- Identification: Dodecamethylcyclohexasiloxane (supplied as Dow Corning® 246 Fluid)
- Lot Number: LL114030
- CAS Number: 540-97-6
- Physical Description: Colorless liquid (as specified in MSDS)
- Source: Dow Corning Corporation
  2200 W. Salzburg Road
  Auburn, MI 48611
- Chemical stability: Stable (as specified in MSDS)
- Storage Conditions: Room temperature (refer to MDMS)
- Expiration Date: March 04, 2004

- Purity: 99.6 %
- Solubility: Soluble in tetrahydrofuran (THF), hexane, acetone, toluene, ethanol (Angelotti , 1991 (6); Varaprath et al., 1998 (7))
- Chemical characterization: HES Study No. 8825, TIS Report No. 1997-I0000-43804
- Archive: A sample is retained in the HES Test Article Archives, Dow Corning Corporation Auburn, MI 48611

LABELED TEST ARTICLE

Chemical identity and radiochemical purity of the labeled test article (^{14}C-D_6) was determined using gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC) with a radioactivity flow-through detector (RAD), respectively (HES study No. 9676). Records of characterization are maintained in the HES Archive. Documentation of study director review was placed in the study records. Any remaining test article was disposed by the study personnel.

- Identification: ^{14}C-Dodecamethylcyclohexasiloxane (^{14}C-D_6)
- Reference Number: 17521-88D
- CAS Number: None
- Physical Description: Colorless liquid (as specified in MSDS)
- Source: Dow Corning Corporation 2200 W. Salzburg road Auburn, MI 48611
- Chemical Stability: Stable (as specified in MSDS)
- Storage Conditions: Freezer (refer to MDMS)
- Expiration Date: March 04, 2004
- Specific Activity: 5.172 µCi/mg
- Radiochemical Purity: 100 %
- Solubility: Soluble in THF, toluene, hexane, acetone, ethanol (Angelotti , 1991(6); Varaprath et al., 1998 (7))
- Chemical characterization: HES Study No. 9676, TIS Report No. 2002-I0000-51536

- Archive: A sample is retained in the HES Test Article Archives, Dow Corning Corporation, Auburn, MI 48611

TEST ARTICLE CARRIER

Corn Oil

- Identification: Corn oil
- CAS number: 8001307
- Lot Number and expiration date: 86H0059 and 02/10/2003
- Physical Description: Yellow, clear liquid
- Source: Sigma, St. Louis, MO
- Chemical stability: Stable at room temperature
- Storage conditions: Room temperature

DOSING SOLUTION

Unlabeled D_6, lot No. LL114030 and ^{14}C-labeled D_6, lot No. 17521-88D were combined and the specific activity of diluted ^{14}C-D_6 was determined to be 0.26 µCi/mg by liquid scintillation analysis. This ^{14}C-D_6 solution was mixed with corn oil to prepare the dosing solution. The purity of the dosing solution was determined by HPLC/RAD and refrigerated for storage.

A single dose of ^{14}C-D_6 was delivered in corn oil. The volume of administered dosing solution was targeted at 10 ml/kg of body weight to deliver a nominal dose of 1000 mg D_6/kg of body weight. Rats in control groups were dosed with corn oil.

D_6 in Corn oil

Homogeneity and eight-days of stability of ^{14}C-D_6 in corn oil were evaluated by extracting/diluting in THF and analyzing by HPLC/RAD, by liquid scintillation counting (LSC), and by gas GC/MS against a THF solvent standard of D_6. These analyses were performed by the HES Analytical Chemistry Group at Dow Corning Corporation.

Specific activity of this dosing solution was determined to be 0.032 µCi/mg on the days of dosing. The D_6 concentration was determined to be 119.89 mg/g of dosing solution. This dosing solution delivered

approximately 320 μCi radioactivity and a nominal dose of 1000 mg D_6/ kg of body weight in 10 ml of dosing solution as outlined in **Table 2**. The dosing solution was refrigerated for storage.

EXPERIMENTAL DESIGN

ROUTE AND RATIONALE OF TEST MATERIAL ADMINISTRATION

Oral administration of the test article was selected as the exposure route since this represents a potential, albeit minor, route for human exposure and is an accepted method of administration of test chemicals in pharmacokinetic and metabolism studies.

ORGANIZATION OF TEST GROUPS

The study consisted of 6 groups being dosed with ^{14}C-D_6 in corn oil and 4 control groups dosed with corn oil. Three experimental approaches were used in this study: 1) Mass balance analysis; 2) Blood kinetic analysis and 3) Qualitative Whole-body autoradiography. Organization of test groups is outlined in **Table 1**. Rats in the MB and WBA exposure groups were dosed on the same day and rats from the BK exposure group were dosed on a separate day.

TEST ARTICLE ADMINISTRATION

Rats in the MB groups (groups 1-4) were placed into the individual glass metabolism cages for the overnight acclimation after their release from quarantine and prior to dosing. On the respective days of dosing all rats were weighed prior to dosing. Dose calculated based on the body weight was delivered using a syringe equipped with a curved stainless steel feeding needle. Immediately after dosing, animals in MB groups were returned to the glass metabolism cages, and animals in WBA and BK groups were returned to the suspended wire-mesh cages for a maximum of 168 hr.

Rats in all exposure groups were dosed with ^{14}C-D_6 in corn oil by oral gavage. The administered dose was determined gravimetrically. An average dose of 1026 mg D_6/kg BW was delivered in approximately 9 g of dosing solution/kg of body weight. Control animals in groups 1, 2 and 7, 8 were dosed with an average of 10 g corn oil/kg of body weight.

At the end of the exposure, rats were euthanized as described in the section Test System: M. Method of Euthanasia.

SAMPLE COLLECTION

Mortality/Morbidity/Daily Observations

All animals were observed in their cages for mortality, morbidity, and signs of distress daily by study personnel through the completion of the in-life phase of the study. Male rat No. D0160 in the control group was removed from the study and replaced with a spare animal No. D0177 due to difficulties during dosing. Cannulated rat No. D0164 appeared distressed during the bleeding process at the 144 hr timepoint. This animal was anesthetized by a 15% Isoflurane in mineral oil emulsion and blood was collected via cardiac puncture after attempting to bleed through the cannula. The study director was notified prior to euthanasia. Rat No. D0161 died in the process of obtaining blood at the 48 hr timepoint. The study director was notified when the animal died.

Blood Kinetics Groups

Immediately after dose administration, cannulated animals in BK groups were returned to wire-mesh cages for collection of blood samples *via* jugular cannula at scheduled time points (15 and 60 min, and 6, 12, 18, 24, 48, 72, 96, 120, 144 and 168 hours). Approximately 300 µL of blood was collected from the jugular cannula. Half of the collected blood sample at each time point (approximately 150 µL) was immediately transferred into a pre-weighed glass vial containing THF, internal standard and glass beads, and processed for analysis of unchanged D_6 concentrations. The extraction efficiency of D_6 from blood was evaluated in a separate study (HES Study No. 9689). The other half of the collected blood sample was immediately transferred into pre-weighed glass vials containing Soluene 350: Isopropanol (IPA) (1:1 v/v) and processed for analysis of total radioactivity. Throughout the conduct of the study, a few animals developed non-patent cannulae at later time points or died due to complications associated with jugular cannula surgery. If no blood was obtained, the sample for that time point was considered lost.

Control Animals (Blood Kinetics)

Blood samples from the control female animals No. D0157 and D0158 and male animal No. D0159 were taken at the 24 hr timepoint. Inadvertently, blood from animal No. D0177 was not collected (see study deviations).

Mass balance groups

Immediately following dose administration, animals in the MB groups were returned to individual glass metabolism cages for collection of urine, feces, CO_2 and expired volatiles at scheduled times as outlined in

Table 3. At the same time, animals in control groups 1 and 2 were placed in glass metabolism cages for collection of the excreta on a daily basis beginning at 24 hr post-dose until 168 hours post-dose.

Glass Metabolism Cage Set-up and Operation

Twelve Roth style glass metabolism cages were set-up and used on the study. Each cage was operated at target conditions and flow rates of 0.5-1 L/min for a minimum of 24 hours prior to housing of animals. During this time the system was evaluated for leaks by monitoring flow rates using flow meters and, if necessary, appropriate actions were taken to assure the leaks were corrected and the system was sealed. In addition, evaluation of Roth style metabolism cage system operation and integrity plus the trapping of volatile ^{14}C-D_6 from the metabolism cages onto charcoal tubes was assessed in the separate study prior to initiation of the exposure period (HES study No. 9674).

Roth style glass metabolism cages were set up and operated in a manner that allowed adequate and uninterrupted airflow. Connections between parts were made using Tygon® tubing. Teflon® tubing was used for connections leading from the exhaust side of the chamber.

Room air was drawn through the cages using a vacuum pump. The airflow rate through each chamber was monitored using a calibrated flow meter and was maintained between 590-700 ml per minute. The room air entering the system was passed through a series of Drierite® and Ascerite® canisters designed to remove H_2O and CO_2, respectively. Cage temperatures were recorded once in the A.M. and once in the P.M. every day during animal housing in the metabolism cages.

Glass tubes containing charcoal were used for trapping expired volatiles. Urine and feces were collected over dry ice and CO_2 was collected in 4N KOH traps (gas towers). Tissues and remaining carcasses were collected at the sacrifice time point.

Excreta Collection

Urine

The Roth style glass metabolism cages that were used have been modified to allow direct collection of urine into glass jars. Jars were pre-weighed and labeled with a minimum of study number, animal number, group number and collection interval. While collecting, each jar was maintained on dry ice. At the appropriate time point, the jars were removed from the cages, capped and placed in an -80°C freezer for storage.

Feces

The Roth style glass metabolism cages that were used have been modified to allow direct collection of feces into glass jars. Jars were pre-weighed and labeled with a minimum of study number, animal number, group number and collection interval. While collecting, each jar was maintained on dry ice. At the appropriate time point, the jars were removed from the cages, capped and placed in an -80°C freezer for storage.

Expired Volatiles

Glass tubes containing charcoal were used for trapping expired volatiles. Glass tubes were supplied closed at each end and were opened by etching and breaking of each end. Each tube was then attached in-line on the exhaust side of the cage. One tube was used per cage, per collection interval. Each tube was labeled with a minimum of study number, animal number, group number and collection interval. At the appropriate time point, the charcoal tubes were removed, capped and placed in -20°C freezer for storage. Charcoal tubes were transferred to the walk-in refrigerator (4± 4°C) to be cracked and desorbed in toluene.

Carbon Dioxide (CO_2)

Cage exhaust air was passed through a glass gas trap filled with 110-153 g of 4N potassium hydroxide (KOH). Each gas trap was filled with KOH prior to initiation of collections. At the appropriate collection interval, KOH was collected into a pre-weighed 8 oz. glass jars labeled with a minimum of study number, animal number, group number and collection interval. Jars with collected KOH were capped and kept refrigerated at 4± 4°C for storage.

Metabolism Cage Rinse

Each glass metabolism cage was rinsed with THF followed by a hexane rinse to remove fecal and urine residues following removal of the animal. The rinse was collected together in a single pre-weighed jar (see study deviations). The cage rinse was kept at 4± 4°C for storage.

Tissues and carcass

At the terminal sacrifice time point (168 hours post-exposure) MB animals were anesthetized using a 15% Isoflurane in mineral oil emulsion. Anesthetizing chambers were prepared by saturating a cotton ball placed in the bottom of a 100 mL beaker with the 15% isoflurane mixture, and a latex glove was stretched over the top to eliminate evaporation. When the rat was anesthetized, the rat's nose was inserted into a opening through the glove covering the beaker. Anesthetized rats were euthanized by exsanguination *via* cardiac puncture. The maximum volume of blood possible was collected into the heparinized Vacutainer® tube until the heart beat and blood flow appeared to stop. Blood collected by open thoracic cardiac

puncture was added into a jar prepared for carcass collection. At this time, liver, lungs, perirenal fat, GI tract, kidney, adrenals, spleen and reproductive organs (ovaries and testis) were collected. Upon removal, the GI tract was mechanically emptied of its contents by squeezing segments of GI tract with hemostats. GI contents were collected into the empty, pre-weighed glass jars. In addition, tools that were used to clean GI tract were rinsed with up to 2 ml of RO water into the jar containing GI contents, as needed and documented in the study records. All tissues were excised, blotted of excess blood, and separately added to a predetermined amount 35% tetraethylammonium hydroxide (TEAH) for solubilization at ambient temperature. Residual carcasses were placed into the pre-weighed jars that were pre-filled with 35% TEAH to be solubilized *in toto* at room temperature. Tissue weights were determined by subtracting tare weights of the jars containing TEAH from total weights after tissues had been added into TEAH.

Control Animals (Mass Balance)

Rats in control groups 1 and 2 were housed in glass metabolism cages under the same environmental conditions as animals in the exposure groups. Excreta collected at the 24 hr time point were used as matrix background. At the terminal sacrifice time point (168 hr post-dose), rats in the control groups were euthanized by exsanguination *via* cardiac puncture under 15% Isoflurane in mineral oil anesthesia and selected tissues and organs were collected as defined in **Table 3**.

Whole Body Autoradiography

Immediately after dose administration, animals in the WBA group were returned to wire-mesh cages for exposure durations of 1, 4, 12, 24, 48, 96 and 168 hr. All animals were observed in their cages for mortality, morbidity, and signs of distress daily by study personnel through the completion of the in-life phase of the study.

At 1, 4, 12, 24, 48, 96 and 168 hr time point animals in Groups 5 and 6 were weighed and sacrificed by CO_2 asphyxiation, and immediately frozen in a hexane/dry ice bath at approximately -75°C and stored at -80 ± 10°C. The frozen carcasses were positioned within a frame and embedded in a 4% aqueous solution of carboxymethylcellulose, which supported the carcass for sectioning. Blocks were stored at -80 ± 10°C until sectioned.

SAMPLE PROCESSING AND ANALYSIS

Blood Kinetics

The blood samples were collected in a vial containing THF, glass beads and the internal standard tetrakis(trimethylsiloxy)silane (M_4Q). The samples were then directly processed by two extractions with THF and an aliquot of the extract was then analyzed for unchanged D_6 content by GC/MS according to the method "Procedure for Determination of D_6 in Biological Matrices (Blood and Feces)" (**Appendix B**). This method was validated prior to the initiation of this study and the results can be found in the study records. Quality control (QC) samples were prepared every day at the same time the samples were processed as a check of the extraction and analysis. QC samples were prepared by spiking varying amounts of D_6 into control blood and processed and analyzed in the same manner as study samples, which were processed for analysis immediately following collection. Parent D_6 was quantified in the blood and QC samples by comparing the extracts of these samples to calibration curves generated from THF solvent standards containing M_4Q and varying amounts of D_6. The various amounts of D_6 in the solvent standards were sufficient to cover the range of concentrations found in the study samples. The standards and samples were analyzed by GC/MS. The mass spectrometer was operated in the electron ionization (EI) and selected ion-monitoring (SIM) modes. The fragment ions of *m/z* 429 and *m/z* 281 were monitored for D_6 and M_4Q quantification, respectively. Remaining details pertaining to the preparation and analysis of blood samples can be found in the procedure in **Appendix B**. Separate aliquots of the blood were taken and solubilized and analyzed for radioactivity content with a liquid scintillation counter. Blood was not further analyzed by HPLC/RAD because of insufficient level of radioactivity ($\leq$ 6410 dpm/g blood).

Control animals (Blood Kinetics)

Blood collected from the control animals at 24 hr time-point was processed and analyzed the same as the samples from the dosed animals. Results obtained from animals in the control groups were used to determine background for radioactivity and parent D_6.

Mass Balance

Radioactivity of all samples collected was quantified by liquid scintillation analysis. Each sample was counted for at least 5 min or a 2 sigma %value of two, whichever came first. All counts were converted to absolute radioactivity (disintegration per minute, dpm) by automatic quench correction. Results were corrected for matrix background radioactivity that was determined by using samples in the control groups.

Urine, KOH and cage washes were weighed following collection and directly analyzed for radioactivity content by LSC. At the same time approximately 1 ml aliquots from urine were removed to new vials for

analysis by HPLC/RAD to determine metabolic profile. Urine samples from 12, 24 and 48 hr time points were selected based on level of radioactivity (approximately >25,000 dpm/g urine). The urine aliquots were centrifuged at approximately (1790-2270) x g in order to remove the insoluble particulates from the urine. Aliquots of the clear urine supernatant were then transferred to auto sampler vials for direct qualitative analysis by HPLC/RAD. The conditions for HPLC/RAD analysis can be found in **Table 11**.

Charcoal tubes that were used to trap expired volatiles were desorbed with toluene (main and back-up portion combined). The charcoal tubes were removed from -20°C freezer and allowed to equilibrate to 4±4°C. The tubes were broken and the contents were placed in pre-weighed glass vials containing approximately 15 ml of a solution of toluene and M_4Q (toluene/ISTD). The charcoal samples were allowed to desorb in toluene for at least 24 hours. Duplicate aliquots of the toluene were taken for total radioactivity analysis by LSC. In addition, aliquots of the charcoal tube extracts were taken for analysis by GC/MS for determination of parent D_6 concentrations according to the method "Procedure for Determination of D_6 in Expired Volatiles (Charcoal Tubes)" (**Appendix B**). Quality control (QC) samples were prepared at the same time as the charcoal tube samples were processed as a check of the extraction and analysis. The QC samples were prepared by spiking varying amounts of D_6 into control charcoal tubes and were processed and analyzed in the same manner as the study samples. Parent D_6 was quantified in the charcoal tube samples and QC samples by comparing the extracts of these samples to calibration curves generated from toluene solvent standards containing M_4Q and varying amounts of D_6. This method was validated prior to the initiation of this study and the results can be found in the study records. Instrumentation and GC/MS methodology were documented and included in the study file.

Feces and contents of GI tract were removed from -80°C frozen storage and allowed to thaw on ice in the closed collection jars. Prior to solubilization and extraction, samples were homogenized with RO water (3:1/water:feces/v:w) supplied by Millipore® system using a tissue homogenizer. Aliquots of the fecal and GI content homogenates were taken and solubilized in 35% TEAH, decolorized with hydrogen peroxide and neutralized with isopropanol. At that time, aliquots of the solubilized feces and GI contents were analyzed by LSC for radioactivity content. On the same day separate aliquots of the homogenates were removed and placed in pre-weighed glass vials and weighed to obtain the homogenate aliquot weight. The homogenate aliquots were then placed back in -80°C freezer until the time of sample processing for parent analysis. Fecal homogenates were extracted and analyzed by GC/MS to determine levels of parent (unchanged) D_6 present in samples according to the method "Procedure for Determination of D_6 in Biological Matrices (Blood and Feces)" (**Appendix B**). This method was validated prior to the initiation of this study and the results can be found in the study records. Instrumentation and GC/MS methodology are documented and included in the study file. The fecal homogenate aliquots were extracted three times with

THF. The method employed the use of an internal standard M_4Q. This internal standard was added with the THF as part of the first extraction. Quality control (QC) samples were prepared at the same time as the feces samples were processed as a check of the extraction and analysis. The QC samples were prepared by spiking varying amounts of D_6 into control fecal homogenate. The QC samples were processed and analyzed in the same manner as the study samples. Parent D_6 was quantified in the fecal and QC samples by comparing the extracts of these samples to calibration curves generated from THF solvent standards containing M_4Q and varying amounts of D_6. The various amounts of D_6 in the solvent standards were sufficient to cover the range of concentrations found in the study samples. The standards and samples were analyzed by GC/MS. The mass spectrometer was operated in the electron ionization (EI) and selected ion-monitoring (SIM) modes. The fragment ions of *m/z* 429 and *m/z* 281 were monitored for D_6 and M_4Q quantification, respectively. Remaining details pertaining to the preparation and analysis of the feces samples can be found in the procedure in **Appendix B**. In addition to parent D_6 quantification by GC/MS, extracts of fecal homogenates from 6, 12, 24, 48 and 72 hour time points that had sufficient radioactivity were further analyzed by qualitative analysis by HPLC/RAD in order to evaluate the metabolite profile of individual radioactive components present in feces. The conditions for the HPLC/RAD analysis can be found in **Table 11**. GI content homogenates were not extracted nor further analyzed by GC/MS because of insufficient level of radioactivity ($\leq$ 173 dpm/g homogenate).

The tissues and carcasses were solubilized using 35%TEAH, and aliquots of the solubilized samples were neutralized with 6N hydrochloric acid and analyzed by LSC to determine radioactivity content.

Control Animals

Samples collected from the control animals were processed and analyzed in the same manner as samples from the exposure groups. Results obtained from control animals were used to determine background levels for radioactivity and parent D_6. Matrix background radioactivity was used to correct radioactivity results for the exposure groups.

Fecal Processing Efficiency (Spiking Experiment)

A spiking experiment was performed after obtaining preliminary mass balance results from the radioactivity measurements for the study. This was done to determine if the fecal processing efficiency was less than 100% under conditions used to analyze fecal samples and to be able to correct the mass balance results for the sample processing efficiency (**Appendix A, Attachment A**). Control feces pellets were placed into the same type of jar as used for collections, and spiked with ^{14}C-D_6 in corn oil to deliver 4.3 - 5.2 µCi /g feces in triplicate (**Table 9**). The spike concentration was targeted to compare with the maximum concentrations found in the fecal samples of the exposure groups. After spiking, the samples were placed in the freezer for

one day. After one day in the freezer the samples were removed and diluted with Milli-Q water and homogenized in the same manner as the study samples. Aliquots were removed from the homogenized samples the same day and solubilized with 35% TEAH and processed for radioactivity measurements. The measured radioactivity was compared to the amount spiked in order to determine a processing efficiency.

Whole Body Autoradiography

Animals from Groups 5 and 6 were sectioned by placing the frozen block on a stage of a Cryomacrocut® microtome (Leica, Deerfield, IL) with temperature maintained at approximately -20 ± 5°C. Sagittal sections of approximately 40 microns in thickness were collected at various levels to include major organs and tissues of interest. The non-dehydrated sections were mounted on a cardboard support, covered with a layer of plastic wrap or mylar, and exposed to Kodak BioMax MR® radiographic film at -80°C for 2 and 4 weeks. One representative section from each level was dehydrated within the cryochamber for 48 to 72 hours and retained as a reference for comparison with the film. At the end of the exposure periods, films were developed on a Cordell™ MXR-14 automatic film processor (Cordell, Peabody, MA). The reported images were digitally acquired from film with a Hewlett-Packard ScanJet Pro (Palo Alto, CA) with output in grayscale at a resolution of 200 PPI (pixels per inch). The films were evaluated for visual clarity and artifacts. These artifacts might occur during preparation, processing or developing due to the physiochemical properties of the test material. All original films were reviewed and evaluated visually for the intensity of radioactivity in tissues/organs relative to background.

Sample Identification and Storage

Samples collected were identified in accordance to the protocol and stored under the following conditions:

KOH	4 ± 4°C
Charcoal tubes	-20 ± 4°C
Toluene extracts	4 ± 4°C
Urine, Feces, GI contents	-80 ± 10°C
Feces and GI contents homogenates	-80 ± 10°C
Solubilized carcass and tissues	Room temperature
THF extracts (blood, feces)	-20 ± 4°C
Cage rinses (THF/Hexane)	4 ± 4°C
Frozen carcasses (WBA)	-80 ± 10°C

DATA ANALYSIS

Parameters evaluated

Radioactivity in excreta and tissues from the MB group is expressed in terms of percent of total radioactivity recovered relative to the amount of administered radioactivity. Radioactivity content was calculated based on the specific activity of the dosing solution and expressed in terms of μg equivalents D_6/g sample. Radioactivity recovered in charcoal tubes (expired volatiles) is reported in μg equivalents D_6/hr. The concentration of parent D_6 (unchanged D_6) in blood and feces is reported in μg D_6/g sample. The concentration of parent D_6 in charcoal tubes is reported in μg D_6/hr. The calculations used to convert from peak areas generated from the GC/MS to μg/g of sample can be found in the procedure in **Appendix B** and the calculation to determine μg/hr in expired volatiles can be found in **Appendix A**. In addition, the qualitative metabolite profile was analyzed in selected feces extracts and urine samples. The metabolites are reported as a percentage of the total radioactivity analyzed.

- Blood kinetics: The radioactivity concentration of D_6 in blood is reported as μg equivalents D_6/g blood, based upon the specific activity of the dosing solution and compared to parent D_6 concentration in blood reported as μg D_6/g of blood. Blood kinetics data are used to generate pharmacokinetics parameters such as blood radioactivity Area Under the Curve (AUC), blood parent D_6 AUC, elimination half-lives ($T_{1/2}$) for radioactivity and parent D_6, maximum blood concentrations (C_{max}) and the time of the peak concentration (T_{max}). Blood radioactivity AUC (expressed as μg equivalents D_6 X hr/g) was compared with blood parent D_6 AUC to evaluate relative metabolism of D_6.
- Absorption: The radioactivity recovered in urine, expired volatiles, expired CO_2, tissues (liver, lungs, perirenal fat, GI tract emptied of it's contents, kidney, adrenals, spleen, ovaries and testes), residual organs and tissues that were left behind in the remaining carcasses of the animals in the MB groups was considered to represent the absorbed portion of administered dose. The radioactivity recovered in urine, expired volatiles and expired CO_2 over 168 hours represent the portion of absorbed dose that was excreted. Radioactivity recovered in the urine and charcoal tubes was compared with the content of parent D_6 to evaluate relative metabolism of D_6 excreted in urine or expired air, respectively.
- Elimination through the GI tract (Dose excreted in feces): The radioactivity recovered in feces over 168 hr, in contents of the GI tract and cage rinses represented the portion of administered dose eliminated through the GI tract. Feces radioactivity was compared with parent D_6 recovered in feces to evaluate relative metabolism of D_6 excreted in feces.

- Limits of quantification: Values that were below limits of quantification (LOQ) were considered equivalent to zero. The LOQ for radioactivity analyses was defined by matrix background radioactivity that was determined by using excreta of control animals collected 24 hr post-dosing, and tissues collected at the sacrifice time point. The LOQ for parent analyses was defined in the study data for each of the matrices.

Statistical analysis

Numerical data obtained during the conduct of the study were processed using Microsoft Excel™ 2000 and subjected to calculation of group mean values and standard error of the mean, where appropriate.

Statistical analysis of the data was carried out in SAS®, v. 8.2 (11,12). Endpoints for statistical analysis included the absorption, disposition and elimination of ^{14}C-D_6 when delivered in corn oil. Gender effect on the endpoints was determined using Analysis of Variance (11). No further multiple comparison tests were used following the Anova test since only the means of only two groups were compared.

The AUC of the time course of radiolabeled and parent D_6 was determined in blood, feces and expired volatiles. Blood radioactivity AUCs were compared to the parent D_6 AUCs for each gender to evaluate relative metabolism of the absorbed D_6. The amount of metabolites in blood was calculated by subtracting the AUC of the parent D_6 from the AUC of the total radioactivity. Elimination half lives ($T_{1/2}$) in blood were calculated as 0.693/K where K is the initial or terminal elimination rate constant. In addition, radioactivity and parent AUCs for feces and expired volatiles were compared for the presence of metabolites. The radioactivity AUCs were compared between males and females. Differences in the radioactivity AUCs between sexes were determined by constructing a 95% confidence interval for the difference ($AUC_{male} - AUC_{female}$) using the method of Nedelman and Jia (13). Confidence intervals for the AUCs were constructed using the method of Nedelman, Gibansky and Lau (14). If the confidence interval did not contain the value zero, then the mean AUCs were considered to be significantly different.

DEVIATIONS

a. A deviation occurred in the protocol with regard to the section X.I (Sample Collection). Aliquot samples of KOH for radioactivity analysis were taken prior to the total KOH weight being recorded. The aliquot weight recorded for each aliquot was added to the weight of the remaining

KOH to determine the total KOH weight. This was considered to have no impact on the study outcome.

b. A deviation occurred in the protocol with regard to the section VII.E (Drinking Water). The main water line was not connected to the animal housing rack for approximately 48 hours. The protocol states that water will be available *ad libitum*. According to the amount of accessible water available in the cage lines and information from the University of Tennessee Health Science Center there was approximately 48 hours of water available to the animals. This was considered to have no impact on the study outcome.

c. A deviation occurred in the protocol with regard to the section X.I. (Sample Collection). Three samples of the GI content were solubilized instead of being homogenized according to the protocol. The three samples were controls, two female and one male rat. The final male control animal was processed correctly and that animal was used for determining GI content background for all exposed rats. This was considered to have no impact on the study outcome.

d. A deviation occurred in the protocol with regard to the section X.I. (Sample Collection). Cage rinses (Hexane and THF) were put into the same jar. The protocol states separate jars will be used. Hexane and THF are miscible and will be counted as a mixture. This was considered to have no impact on the study outcome.

e. A deviation occurred in the protocol with regard to the section X.I. (Sample Collection). Blood was drawn from the male control animal No. D0160 that was excluded from the study due to dosing problems, instead of the male control animal No. D0177 at the 24-hour time point. These data were not used and only blood data from other male control animal No. D0159 were used to determine background levels. This was considered to have no impact on the study outcome.

f. A deviation occurred in the protocol with regard to section X.I.g (Whole Body Autoradiography). Animals No. D0147 and D0154 were kept in a hexane/dry ice bath for 68 and 64 minutes, respectively instead of approximately 20-40 minutes, as stated in the protocol. This was considered to have no impact on the study outcome.

g. A deviation occurred in the protocol with regard to section VIII. (Animal Welfare Act Compliance). Animals for the study were ordered before LACUC written approval was received. However, protocol and protocol certification were circulated before animals were ordered. This was considered to have no impact on the study outcome.

h. A deviation occurred in the protocol with regard to GC/MS procedure for determination of parent D6 in blood and feces. All standards, QC samples and samples were run at initial oven temperature of 80°C for 9.67 minutes instead of initial temperature of 70°C and total run time of 13.57 min. This was considered to have no impact on the study outcome.

RESULTS

The disposition of ^{14}C-D_6 was evaluated in male and female rats following a single oral dose of 1000 mg D_6 in corn oil/kg body weight. A group of animals cannulated *via* jugular vein were used to obtain estimates of blood concentrations. A separate group of rats dosed with ^{14}C-D_6 in corn oil was placed in glass metabolism cages for quantitative assessment of ^{14}C-D_6 excretion and absorption. Finally, WBA was used for qualitative *in vivo* assessment of tissue distribution of radioactivity.

Blood Kinetics

This portion of the study was designed to provide data on blood kinetics for both male and female rats when dosed with D_6 in corn oil. Individual dosing data are presented in **Table 2**. Total radioactivity (μg equivalents D_6/g of blood) and parent D_6 concentrations (μg D_6/g) are presented in **Table 4** and **Figure 1**. The corresponding individual animal values are presented in **Appendix A**.

Blood Quantification

For parent D_6 analysis the LOQ (expressed as μg of D_6 in the solvent extract), for each analysis was determined by multiplying 10 times the standard deviation of three solvent blanks or was determined to be the value of the lowest prepared solvent standard to meet acceptable accuracy (% relative error from prepared concentration) within ± 15%. For blood analysis the LOQ was determined by taking 10 times the standard deviation of the three solvent blanks. The LOQ was approximately 0.092 μg D_6 present in the blood extracts. The LOQ expressed as μg D_6/g of blood depended on the individual sample size of the blood obtained and averaged 0.447 μg D_6/g of blood. The individual results from the analysis of the blood quality control samples (QC spikes) analyzed daily with the daily collection of the blood samples are presented in **Appendix A**. The QC spikes were prepared at three levels to bracket expected levels in blood (approximately 40, 800, and 4000 ng). The lowest level of QC spikes did not meet acceptance criteria of within 20% of expected. This was due to the LOQ being greater than twice what was expected for the lowest QC level prepared. The middle and upper levels were all within acceptance criteria for time points (15 min through 72 hour) where samples were quantified. All samples analyzed from 96 hour through 168 hour were below limit of quantification (BLQ).

Pharmacokinetic parameters

Basic pharmacokinetic parameters were determined from the blood curves. The area under the curve analysis for total radioactivity and parent D_6 in blood as well as values for T_{max}, C_{max} and $T_{½}$ are presented in **Table 5**. Statistical analysis **(Appendix C)** showed significant difference between radioactivity and

parent AUCs for both males and females when D_6 was administered in corn oil indicating presence of metabolites in blood ($AUC_{metabolites} = AUC_{radioactivity} - AUC_{parent}$). The radioactivity and parent AUC in females was 293.53 µg equivalent D_6 X hr/g and 177.20 µg D_6 X hr /g, respectively. The radioactivity and parent AUC in males was 225.93 µg equivalent D_6 X hr/g and 108.36 µg D_6 X hr /g, respectively. The parent AUC was significantly larger for females compared to males (177.20 vs. 108.36 µg D_6 X hr/g) based on statistical analysis. The radioactivity AUC was also significantly larger for females compared to males (293.53 vs. 225.93 µg equivalent D_6 X hr/g) based on statistical analysis (**Figure 1** and **Appendix C**). However, statistical difference between genders was not considered to be biologically significant considering the variability and accuracy at such low blood levels of D_6 in both males and females.

Maximum concentration (C_{max}) of total radioactivity and parent D_6 was achieved 6 hr after dosing for D_6 in corn oil. Maximum radioactivity concentration in blood was 6.60 and 6.80 µg equivalents D_6/g, for males and females, respectively. Maximum parent concentration in blood was 6.38 and 6.10 µg D_6/g for males and females, respectively (**Table 5**). The initial radioactivity elimination half- life ($T_{1/2}$') was 15.31 hr and 25.22 hr for males and females, respectively. Terminal radioactivity elimination half-life ($T_{½}$") was 104.77 hr and 117.62 hr for males and females, respectively (**Table 5**). All of the parent D_6 was eliminated initially and there was no detectable D_6 in blood after the 24-hr time point in males and 72-hour time point in females. The parent elimination half-life was 8.55 hr and 18.93 hr for males and females, respectively. Blood was not further analyzed by HPLC/RAD because of insufficient levels of radioactivity (≤ 6408 dpm/g of blood).

Mass Balance

Following a single oral dose of ^{14}C-D_6 in corn oil, animals were placed in glass metabolism cages for collection of expired volatiles, CO_2, urine, and feces at predetermined time points as outlined in **Table 3**. Individual dosing data are presented in **Table 2**. At the terminal sacrifice time point (168 hours post-dosing) liver, lungs, perirenal fat, kidneys, adrenals, spleen, emptied GI tract, contents of GI tract, reproductive organs (ovaries and testis) and remaining carcasses were collected.

The radioactivity content was measured in all samples and D_6 concentrations (µg equivalents D_6/ g of sample) were calculated based upon specific activity of administered dosing solution. Total dose recovered was ≥ 95% in both sexes (**Table 6**). Data showed that most of the administered dose (≥ 83%) was excreted in feces. Percent dose recoveries in feces were corrected for the sample-processing efficiency associated with analysis of the fecal samples. Fecal processing efficiency was determined to be 87% in a spiking experiment and was used to correct mass balance results (**Table 9** and **Appendix A**). The discrepancy in

processing efficiency from 100% was possibly due to the experimental difficulties and loss of D_6 when taking aliquots from the aqueous fecal homogenate sample containing highly lipophilic D_6 that tends to migrate to the glass jar rather than stay in the aqueous sample. Approximately 12% of administered dose appeared to be absorbed (radioactivity recovered in urine, expired volatiles, expired CO_2, tissues and carcass) with the majority (~ 95%) of absorbed dose found in expired volatiles (**Table 6**). Data indicated that expired volatile and consequently absorption values might be falsely elevated due to possible off gassing of ^{14}C-D_6 from the fecal pellets that were not collected in collection jars as intended but remained inside the cage (see Concluding Discussion) because of altered consistency of fecal material caused by high dose of corn oil.

Elimination through GI tract (Dose excreted in feces)

The majority of administered dose was excreted in feces regardless of sex (**Table 6, Figure 2**). Cumulative elimination of radioactivity through the GI tract during 168 hrs post-dosing was expressed as percent of administered dose (± standard error of the mean) in feces, contents of the GI tract and cage rinses used to remove residual feces. Radioactivity eliminated through the GI tract was determined to be 84.78 ± 5.21% and 82.83 ± 6.81% of administered dose for male and female rats, respectively. Cumulative percent dose recovered in feces was not significantly different between sexes. The majority of administered dose was excreted in feces within 48 hours (**Figure 7, Appendix A**).

Feces Quantification

In addition to radioactivity content, fecal samples were analyzed by GC/MS for the levels of parent D_6. Average total radioactivity and parent D_6 concentrations in feces per time point are presented in **Table 8**.

The limit of quantification for each analysis of feces samples was determined to be the value of the lowest prepared solvent standard to meet acceptable accuracy (% relative error from prepared concentration) within ± 15%. The LOQ was approximately 0.016 µg D_6 present in the fecal extract. The LOQ expressed as µg D_6/g of feces depended on the individual amount of feces obtained and averaged 0.234 µg D_6/g feces. The individual results from the analysis of the feces quality control samples (QC spikes) analyzed along side the feces samples are presented in **Appendix A, Attachment C**. All the feces QC spikes had accuracies within 20% of the prepared concentrations except for one QC sample at 27%. This sample was also at the lowest QC concentration spiked at approximately 0.031 µg.

The radioactivity and parent D_6 concentrations in feces from male and female rats dosed with D_6 in corn oil are presented as group means with standard errors of the means in **Table 8.** Graphical representations of

these concentrations as functions of post-exposure time are presented in **Figure 3**. The corresponding individual animal values for these parameters are presented in **Appendix A**. Statistical analysis (**Appendix C**) showed that there was no significant difference between radioactivity AUC and parent AUC in feces when D_6 was administered in corn oil (**Figure 3**).

Fecal Metabolite Profile

The feces samples collected at 6, 12, 24, 48 and 72 were analyzed by HPLC/RAD under analysis conditions presented in **Table 11** in order to evaluate the metabolite profile of individual radioactive components present in feces. **Figure 6** shows a representative chromatogram of 24-hour fecal sample from male and female rats dosed with D_6 in corn oil. Samples of GI contents homogenates were not further analyzed by GC/MS or by HPLC/RAD because of insufficient level of radioactivity in the homogenates. The majority of the radioactivity present in all the feces extracts corresponded to parent D_6 based on the retention time comparisons (retention time ~45 minutes). Some of the fecal samples had a small metabolite peak present that is yet unidentified and that eluted fairly close to parent D_6 indicating that it is relatively non-polar (retention time ~ 44 minutes). This peak was present randomly in some of the samples regardless of gender, indicating that the metabolite presence was not due to gender differences (**Table 12**).

Absorption

Absorption of radioactivity, expressed as percent of administered dose (± standard error of the mean) in urine, expired volatiles, expired CO_2, tissues and carcasses, was determined to be 11.88 ± 1.84% and 11.83 ± 4.79% for male and female rats, respectively (**Table 6**). Statistical analysis of endpoints showed only significantly higher level of radioactivity in adrenals of females (0.001 vs. 0.000%), emptied GI tract of females (0.007 vs. 0.005%) and CO_2 traps of males (0.125% vs. 0.093%) as shown in **Appendix C**. These observations were not considered to be biologically significant due to the very low levels of radioactivity, just above the background, found in samples of both males and females.

The majority of the absorbed dose was found in charcoal tubes (11.20 ± 1.83% and 11.21 ± 4.79% of administered dose for male and female rats, respectively) as shown in **Table 7**. However, considerable variability was seen in radioactivity levels in expired volatiles (from 3.86 % to 25.28% of administered dose) that maybe due to off gassing from the fecal pellets that were not collected as intended but remained inside the cage (see Concluding Discussion). This could potentially give false high values for expired volatiles and absorption due to partitioning from the fecal matter into the air. In addition to radioactivity content, charcoal tubes were analyzed by GC/MS for parent D_6 (**Appendix A**). Comparison of radioactivity and parent AUCs for D_6 in corn oil showed that all of the radioactivity trapped in the charcoal tubes (expired volatiles) could be attributed to the parent D_6 (**Figure 4**). Average total radioactivity

(μg Equivalents D_6/hr) and parent D_6 concentrations (μg D_6/hr) in expired volatiles are presented in **Table 10**. Less than 1% of administered dose was found in urine, tissues and remaining carcass, and CO_2 traps (KOH) combined. The corresponding individual animal values for these parameters are presented in **Appendix A**.

Expired Volatiles Quantification

The limit of quantification for each analysis of charcoal tube samples was determined to be the value of the lowest prepared solvent standard to meet acceptable accuracy (% relative error from prepared concentration) within ± 15%. The LOQ was approximately 0.024 μg D_6/g of toluene extract. The LOQ expressed as μg D_6/hr depended on the individual amount of toluene used to extract the charcoal tube and number of hours that the sample was collected and averaged 0.013 μg D_6 in expired volatiles/hr. The individual results from the analysis of the charcoal tube quality control samples (QC spikes) analyzed along side the charcoal tube samples are presented in **Appendix A, Attachment C**. All the charcoal tube QC spikes had accuracies within 20% of the prepared concentrations except for one QC sample at 41%. This sample was also at the lowest QC concentration spiked at approximately 0.109 μg.

Urine Metabolite Profile

In addition to radioactivity content (0.32 and 0.38% of administered dose in females and males, respectively, **Table 7**) urine samples collected 12, 24 and 48 hr were directly analyzed by HPLC/RAD under analysis conditions presented in **Table 11** to determine metabolic profile. The metabolic profiles of urine were compared qualitatively. The radioactivity eliminated in the urine consisted entirely of polar metabolites of D_6. No parent D_6 was found in any of the urine samples regardless of gender at any of the time points investigated. The mean results from the qualitative urinary metabolite profiles can be found in **Table 13**. This table shows the mean percent of radioactivity that can be attributed to individual metabolites from urine at 12, 24 and 48 hours following exposure. There were 2 major metabolites common to all of the rats regardless of gender at these time points. These metabolites have been identified as methylsilanetriol [$CH_3Si(OH)_3$] and dimethylsilanediol [$(CH_3)_2Si(OH)_2$] based on retention time comparison to urinary metabolite profiles performed previously in a separate study by Varaprath *et.al.* (8). No confirmation of identity was conducted within this study. **Figure 5** shows representative chromatograms of 24-hour urine samples from male and female rats dosed with D_6 in corn oil. Individual animal results from the qualitative metabolite profile analysis can be found in **Attachment B** of **Appendix A**. Data showed that methylsilanetriol represented approximately 55-70% of urine metabolites in males and approximately 50% of urine metabolites in females. Dimethylsilanediol represented approximately 30-40% of urine metabolites in males and approximately 50% of urine metabolites in females.

Whole-Body Autoradiography

The WBA portion of this study was performed concurrently with the mass balance and blood kinetic analysis following single, oral dose of ^{14}C-D_6 in corn oil. Individual dosing data are presented in **Table 2**. All original films were reviewed and evaluated visually for the intensity of radioactivity as compared to background (**Appendix D**). Figures in this report are scanned images of the original autoradiographs (**Figures 9, 10**, and **Appendix D**). It should be noted that at the earlier time points (1 through 24 hours) the high intensity of radioactivity in the gastrointestinal tract tended to obscure adjacent organs rendering difficulty in visualization. Qualitative assessment of tissue distribution by WBA showed that the majority of radioactivity was concentrated in the contents of the GI tract, and eliminated within 48 hours. Low to moderate levels of radioactivity were seen in organs and tissues such as liver, brown fat and bone marrow indicating some absorption of D_6. Both sexes showed comparatively similar patterns of disposition at each time point (**Figure 9** and **Figure 10**) with decreasing intensity of radioactivity over time and only low levels of radioactivity observed at the 96 and 168 hr time points.

One hour following dosing the highest concentration of radioactivity in the female rat was found in the contents of the stomach and small intestines. In the male, the highest concentrations were also found in the contents of the stomach and small intestines, as well as the ethmoturbinates and hard palette. At 4, 12 and 24 hr post-dose, the highest concentrations for both the female and male were seen in contents of the GI tract (stomach, cecum, small intestines and colon). Low radioactivity was detected in brown fat, bone marrow, adrenal cortex, esophagus, and myocardium. Low to moderate radioactivity was detected in the liver and on the skin surface. Radioactivity on the skin surface is most likely due to fecal contamination. Radioactivity detected on hard palette, esophagus and ethmoturbinates can be attributed to the contamination during oral dosing.

At 48 hr post-dose the intensity of radioactivity in tissues and organs decreased significantly. A moderate amount of radioactivity observed at 48 hr for the female was in the adrenal cortex, wall of the stomach, and the contents of the cecum and colon. A low amount was found in the brown fat, liver, and myocardium. The male had a moderate level in the contents of the cecum and colon and a low amount in the liver, brown fat, myocardium, and adrenal cortex.

At 96 hours, the female (**Figure 9**) had low amounts of radioactivity in the brown fat, liver, bone marrow, myocardium, and adrenal cortex. The male (**Figure 10**) had low levels in the brown fat.

At 168 hours, the last time point, both female and male (**Figures 9** and **10**) had moderate amounts of radioactivity in the brown fat. Both had low levels (slightly above background) in the liver, bone marrow, and myocardium.

Whole-body autoradiography data supported mass balance data showing that the majority of administered D_6 in corn oil stayed in the GI tract and was excreted in feces within 48 hr. Low levels of radioactivity were detected in organs and tissues indicating some absorption of D_6. No significant radioactivity was seen in the respiratory tract at any time point.

CONCLUDING DISCUSSION

Mass balance data indicated that approximately 12% of administered dose was absorbed (radioactivity recovered in urine, expired volatiles, expired CO_2, tissues and carcass) with the majority of absorbed dose found in expired volatiles. Expired volatiles represented approximately 95% of total absorption in both sexes (**Table 7**). Entire radioactivity in the expired volatiles was attributed to unchanged ^{14}C-D_6. Data also showed that highest radioactivity concentrations in expired volatiles traps (charcoal tubes) occurred concurrently with the highest concentrations in the excreted fecal material (**Figure 7** and **8**). This observation indicated that evaporation from the fecal matter, that was not collected as intended and remained inside the cage, might have contributed to the high levels of radioactivity trapped in the charcoal tubes. The design of the cages is intended for normal fecal pellets. The intent is for the fecal pellet to hit the side of the cage and drop into the collection jar that is stored over dry ice. This prevents any of the volatile test material in the feces to escape. However, it is possible that corn oil at the high dose levels of approximately 10 ml/kg of body weight affected consistency of fecal material causing difficulties in it's collection and removal. It was noticed that some of fecal pellets were not completely removed from the main body of the cage. For example, an observation was made for a rat No. D0138 that fecal pellets were caught inside the body of the cage, which most likely contributed to the significantly high recoveries in the charcoal tubes (25% of administered dose) and consequently low dose recovery in feces (65% of administered dose). Given that the majority of the orally administered dose was eliminated in feces (**Table 6**), and that any volatiles that might have escaped from fecal material, which was not frozen and captured as intended, would be trapped in the same charcoal tubes that were used to collect expired volatiles which could potentially give false high values for expired volatiles and consequently high absorption values due to partitioning from the fecal matter into the air.

Results of a follow-up experiment (HES Study No. 9748) demonstrated that evaporation of ^{14}C-D_6 from the fecal matter that was not frozen and captured in collection jars but remained inside the body of the cage

throughout the experiment could significantly contribute to the radioactivity trapped in the charcoal tubes giving false high values for expired volatiles and absorption of D_6.

SUMMARY OF RESULTS

The majority of administered ^{14}C-D_6, regardless of sex, was excreted in feces unchanged within 48 hours. Approximately 12% of ^{14}C-D_6 delivered in corn oil appeared to be absorbed after single oral administration in Fischer 344 rats. Both sexes showed similar patterns of disposition with the majority of absorbed dose excreted in expired volatiles. Possible escape of ^{14}C-D_6 from the fecal pellets that was not collected as intended, but remained inside the cage, might contribute to the percent dose recovered in expired volatiles. Statistical analysis of the blood curves indicated presence of small level of metabolites in the blood Qualitative assessment of tissue distribution (WBA) showed that only low level of radioactivity was systemically available and distributed to organs and tissues such as liver, brown fat and bone marrow.

ARCHIVE

Protocol, amendments and deviations, study authorization form, raw data, correspondence and final report, at minimum, are retained in the HES archives, Dow Corning Corporation, Auburn, MI 48611.

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Table 1. Organization of Test Groups

Group ID	Number of Animals/ Sex	Treatment	Target Dose (mg D_6/ kg body weight)	Volume of Dosing Solution (ml dosing solution/kg body weight)	Concentration of Dosing Solution (mg D_6/ml dosing solution)	Exposure Duration/ Sacrifice Time Point
1 Mass Balance control	2 Females	Corn oil	0	10	0	168 hr
2 Mass Balance control	2 Males	Corn oil	0	10	0	168 hr
3 Mass Balance	4 Females	^{14}C-D_6 in Corn oil	1000	10	100	168 hr
4 Mass Balance	4 Males	^{14}C-D_6 in corn oil	1000	10	100	168 hr
5 Whole Body Autoradiography	7 Females	^{14}C-D_6 in Corn oil	1000	10	100	up to 168 hr
6 Whole Body Autoradiography	7 Males	^{14}C-D_6 in Corn oil	1000	10	100	up to 168 hr
7 Blood Kinetics control	2 Females Cannulated	Corn oil	0	10	0	24 hr[1)]
8 Blood Kinetics control	2 Males Cannulated	Corn oil	0	10	0	24 hr[1)]
9 Blood Kinetics	6 Females Cannulated	^{14}C-D_6 in Corn oil	1000	10	100	168 hr
10 Blood Kinetics	6 Males Cannulated	^{14}C-D_6 in Corn oil	1000	10	100	168 hr

[1)] Control blood from animals in group 7 and 8 was collected at 24 hr - time point.

Table 2. Individual Dosing Data

Mass Balance

Animal ID	Group	Sex	Body weight (g)	Dosing solution (g) / animal	Dosing solution (g/kg Body Weight)	mg D_6 per animal	mg D_6 per kg Body Weight)	Average mg D_6 per kg Body Weight
D0131	1 Control	Female	151.1	1.4197	9.40	0	0	
D0132	1 Control	Female	150.9	1.4323	9.49	0	0	
D0133	2 Control	Male	200.6	1.8792	9.37	0	0	
D0134	2 Control	Male	194.6	1.8717	9.62	0	0	0
D0135	3	Female	149.2	1.2770	8.56	153.10	1026.14	
D0136	3	Female	152.6	1.2519	8.20	150.09	983.55	
D0137	3	Female	148.0	1.2388	8.37	148.52	1003.51	
D0138	3	Female	151.0	1.2279	8.13	147.21	974.92	997.03
D0139	4	Male	194.5	1.6849	8.66	202.00	1038.57	
D0140	4	Male	182.4	1.4804	8.12	177.49	973.05	
D0141	4	Male	191.0	1.6267	8.52	195.03	1021.07	
D0142	4	Male	201.2	1.7443	8.67	209.12	1039.38	1018.02

Whole Body Autoradiography

Animal ID	Group	Sex	Body weight (g)	Dosing solution (g) / animal	Dosing solution (g/kg Body Weight)	mg D_6 per animal	mg D_6 per kg Body Weight)	Average mg D_6 per kg Body Weight
D0143	5	Female	149.8	1.2433	8.30	149.06	995.05	
D0144	5	Female	147.1	1.1885	8.08	142.49	968.66	
D0145	5	Female	150.1	1.2772	8.51	153.12	1020.14	
D0146	5	Female	151.0	1.2804	8.48	153.51	1016.60	
D0147	5	Female	149.5	1.2982	8.68	155.64	1041.08	
D0148	5	Female	150.1	1.2804	8.53	153.51	1022.70	
D0149	5	Female	155.0	1.3515	8.72	162.03	1045.36	1015.66
D0150	6	Male	193.5	1.6664	8.61	199.78	1032.48	
D0151	6	Male	201.3	1.7419	8.65	208.84	1037.44	
D0152	6	Male	205.6	1.7235	8.38	206.63	1005.01	
D0153	6	Male	211.4	1.7671	8.36	211.86	1002.16	
D0154	6	Male	211.0	1.8424	8.73	220.89	1046.85	
D0155	6	Male	219.4	1.8135	8.27	217.42	990.98	
D0156	6	Male	216.7	1.8026	8.32	216.11	997.29	1016.03

Blood Kinetics

Animal ID	Group	Sex	Body weight (g)	Dosing solution (g) / animal	Dosing solution (g/kg Body Weight)	mg D_6 per animal	mg D_6 per kg Body Weight)	Average mg D_6 per kg Body Weight
D0157	7 Control	Female	136.6	1.4128	10.34	0	0	
D0158	7 Control	Female	141.0	1.3647	9.68	0	0	
D0159	8 Control	Male	162.9	1.5894	9.76	0	0	
D0177	8 Control	Male	172.1	1.7427	10.13	0	0	0
D0161	9	Female	132.7	1.1729	8.84	140.62	1059.68	
D0162	9	Female	137.5	1.1629	8.46	139.42	1013.96	
D0163	9	Female	139.2	1.1243	8.08	134.79	968.34	
D0164	9	Female	139.6	1.1808	8.46	141.57	1014.08	
D0165	9	Female	140.5	1.2364	8.80	148.23	1055.03	
D0166	9	Female	142.0	1.2916	9.10	154.85	1090.49	1033.60
D0167	10	Male	169.9	1.4934	8.79	179.04	1053.82	
D0168	10	Male	171.3	1.5176	8.86	181.95	1062.14	
D0169	10	Male	173.6	1.5599	8.99	187.02	1077.28	
D0170	10	Male	176.1	1.5062	8.55	180.58	1025.43	
D0171	10	Male	176.2	1.6538	9.39	198.27	1125.28	
D0172	10	Male	181.5	1.6205	8.93	194.28	1070.42	1069.06

Average dose in all exposure groups was 1026 mg D_6/ kg Body Weight
D_6 concentration in corn oil dosing solution: 119.89 mg/g of dosing solution

Table 3. Sample Collection Time Points

Groups	Exposure Duration (Hour)	Expired Volatiles (Hour)	Urine (Hour)	Feces (Hour)	CO_2 (Hour)	Tissues / Carcasses (Hour)	Blood (Hour)
Mass Balance 1, 2	168	24, 48, 72, 96, 120, 144, 168	24, 48, 72, 96, 120, 144, 168	24, 48, 72, 96, 120, 144, 168	24, 48, 72, 96, 120, 144, 168	168[1)]	NA
Mass Balance 3, 4	168	1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144, 168	6, 12, 24, 48, 72, 96, 120, 144, 168	6, 12, 24, 48, 72, 96, 120, 144, 168	24, 48, 72, 96, 120, 144, 168	168[1)]	NA
Whole Body Autoradiography 5,6	up to 168	N/AP	N/AP	N/AP	N/AP	1, 4, 12, 24, 48, 96, 168[2]	N/AP
Blood Kinetics 7,8	24	N/AP	N/AP	N/AP	N/AP	N/AP	24
Blood Kinetics 9,10	168	N/AP	N/AP	N/AP	N/AP	N/AP	0.25, 1, 6, 12, 18, 24, 48, 72, 96, 120, 144, 168

N/AP = Not Applicable

[1)] Tissues collected in Mass Balance groups at sacrifice time points: liver, lungs, perirenal fat, emptied GI tract, adrenals, kidneys, spleen and reproductive organs (ovaries and testes).

[2)] Whole-Body Autoradiography: Carcasses collected at sacrifice time points, frozen, embedded in 4% carboxymethylcellulose, and blocks stored at -80°C.

Table 4. Radioactivity *vs.* Parent D_6 Concentration in Blood of Fischer 344 Rats Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

	Females			Males		
Time point (hour)	Radioactivity (µg Eq. D_6/g ±SEM)	Parent (µg D_6/g ±SEM)	N	Radioactivity (µg Eq. D_6/g ±SEM)	Parent (µg D_6/g ±SEM)	N
0.25	0.08 ± 0.02	BLQ N/AP	6	0.07 ± 0.02	BLQ N/AP	6
1	0.79 ± 0.10	0.64 ± 0.09	6	0.81 ± 0.05	0.49 ± 0.03	6
6	6.80 ± 0.90	6.10 ± 0.55	6	6.60 ± 0.63	6.38 ± 0.34	6
12	6.41 ± 0.44	4.96 ± 0.33	6	6.49 ± 0.38	4.97 ± 0.39	6
18	4.42 ± 0.48	4.15 ± 0.57	6	3.97 ± 0.26	3.45 ± 0.20	6
24	3.73 ± 0.31	2.87 ± 0.58	6	2.78 ± 0.14	1.42 ± 0.13	6
48	2.22 ± 0.31	1.34 ± 0.32	6	1.09 ± 0.10	BLQ N/AP	6
72	0.99 ± 0.09	0.50 ± 0.07	4	0.68 ± 0.04	BLQ N/AP	6
96	0.82 ± 0.05	BLQ N/AP	4	0.63 ± 0.05	BLQ N/AP	5
120	0.69 ± 0.04	BLQ N/AP	3	0.47 ± 0.01	BLQ N/AP	5
144	0.52 ± 0.09	BLQ N/AP	2	0.39 ± 0.04	BLQ N/AP	3
168	0.61 ± 0.04	BLQ N/AP	4	0.39 ± 0.04	BLQ N/AP	6

Difficulties with bleeding animals through the jugular vein cannulae and with cannula patency caused decrease in number of samples (N) over time.

BLQ = Below Limit of Quantification
N/AP = Not Applicable

Table 5. Summary of Pharmacokinetic Parameters in Blood from Fischer 344 Rats Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

Pharmacokinetics Parameters	Females	Males
Radioactivity AUC (µg Eq. ^{14}C-D_6 X hour/g)	293.531†+	225.934†+
C_{max} (µg Eq. ^{14}C-D_6/g)	6.80	6.60
T_{max} (hour post-dosing)	6	6
T ' 1/2 (hour) Initial Elimination	25.22	15.31
T " 1/2 (hour) Terminal Elimination	117.62	104.77
Parent AUC µg D_6 X hour/g	177.198†*	108.362†*
C_{max} (µg D_6/g)	6.10	6.38
T_{max} (hour post-dosing)	6	6
T ' 1/2 (hour) Initial Elimination	18.93	8.55
T " 1/2 (hour) Terminal Elimination	N/AP	N/AP

†Statistically significant difference between parent D_6 and radioactivity at α=0.05
+Statistically significant difference between sexes in radioactivity at α=0.05
* Statistically significant difference between sexes in parent D_6 at α=0.05

N/AP = Not applicable because the levels of parent D_6 were BLQ after the 24 hour time point in males and after 72 hour time point in females.

Table 6. Disposition of Radioactivity in Fischer 344 Rats Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

N/Sex	Dosing Solution	Average Dose (mg D_6/kg Body Weight)	Absorbed [1] (% Dose)	Eliminated through GI Tract [2), 3)] (% Dose)	Total Recovered (% Dose)
4 / Females	D_6 in Corn Oil	997.03	11.83 ± 4.79	82.83 ± 6.81	94.66 ± 3.40
4 / Males	D_6 in Corn Oil	1018.02	11.88 ± 1.84	84.78 ± 5.21	96.65 ± 4.46

[1] Absorbed radioactivity = Percent of administered dose recovered in urine, expired volatiles, CO_2, tissues and remaining carcass

[2] Radioactivity excreted in feces = Percent of administered dose recovered in feces, contents of gastrointestinal tract and cage rinse

[3] Percent dose recovered in feces was corrected for the fecal sample processing efficiency: 87%

Table 7. Disposition of Absorbed Radioactivity in Fischer 344 Rats Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

Percent of Administered Dose ± Standard Error of the Mean

N/Sex	Dosing Solution	Urine (% Dose)	Expired Volatiles (% Dose)	CO_2 (% Dose)	Tissues [1] (% Dose)	Remaining Carcass (% Dose)	Total Absorbed [2] (% Dose)
4 / Female	D_6 in Corn Oil	0.32± 0.02	11.21± 4.79	0.09± 0.01	0.04± 0.00	0.17± 0.01	11.83± 4.79
4 / Male	D_6 in Corn Oil	0.38± 0.02	11.20± 1.83	0.13± 0.00	0.03± 0.00	0.14± 0.01	11.88± 1.84

[1] Tissues collected at the sacrifice time point (168 hour pose dosing): liver, lungs, sample of perirenal fat, emptied GI tract, kidneys, adrenals, spleen and reproductive organs (ovaries or testis)

[2] Total Absorbed = Percent of administered dose recovered in urine, expired volatiles, CO_2, tissues and remaining carcass.

Table 8. Radioactivity *vs.* Parent D_6 Concentration in Feces of Fischer Rats Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

Females				Males		
Time point (hour)	**Radioactivity (µg Eq. D_6/g ±SEM)**	**Parent (µg D_6/g ±SEM)**	**N**	**Radioactivity (µg Eq. D_6/g ±SEM)**	**Parent (µg D_6/g ±SEM)**	**N**
6	194.98± 192.93	185.75± 183.92	4	1.44± 0.28	0.64± 0.36	4
12	8175.22± 2532.16	6240.57± 1212.88	4	5389.31± 3618.18	4076.88± 2510.22	4
24	27290.60± 4101.46	21914.40± 721.30	3	23638.24± 1693.31	22502.86± 1258.12	4
48	7960.98± 1532.00	8956.40± 1775.59	4	12409.59± 1982.12	13432.38± 2940.21	4
72	578.81± 184.09	597.76± 207.68	4	440.54± 259.62	432.44± 256.95	4
96	27.02± 6.51	25.26± 6.41	4	32.33± 20.36	30.67± 21.60	4
120	8.59± 2.46	6.71± 2.36	4	4.40± 1.19	2.40± 1.24	4
144	3.69± 0.78	2.57± 1.32	4	2.73± 0.27	0.52± 0.10	4
168	2.70± 0.46	1.22± 0.43	4	2.26± 0.35	0.45± 0.02	4

Table 9. Results from Fecal Processing Efficiency Experiment

Sample ID	µCi/g Feces Recovered	µCi/g Feces spiked	% Processing Efficiency	Average % Processing Efficiency
Corn Oil 1 (09/25/02)	5.02	5.69	88%	87%
Corn Oil 2 (10/1/02)	4.32	5.20	83%	
Corn Oil 3 (10/1/02)	5.20	5.86	89%	

Table 10. Radioactivity *vs.* Parent D_6 Concentration in Charcoal Tubes (Expired Volatiles) of Fischer 344 Rats Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

	Females			Males		
Time point (hour)	Radioactivity (µg Eq. D_6/g ±SEM)	Parent (µg D_6/g ±SEM)	N	Radioactivity (µg Eq. D_6/g ±SEM)	Parent (µg D_6/g ±SEM)	N
1	98.21± 52.78	82.57± 44.76	4	124.01± 65.20	105.22± 55.87	4
2	41.16± 27.35	35.34± 23.32	4	61.14± 46.80	53.53± 40.20	4
4	11.64± 7.56	15.23± 11.04	4	21.45± 15.97	22.53± 17.20	4
6	36.38± 31.06	35.49± 30.46	4	7.79± 5.37	6.63± 4.78	4
12	380.09± 299.84	386.66± 268.47	4	241.50± 69.24	298.04± 92.72	4
24	780.93± 222.94	696.02± 202.67	4	999.34± 188.55	888.99± 165.44	4
48	127.96± 60.89	136.79± 61.90	4	302.66± 50.53	321.66± 32.94	4
72	34.55± 24.01	30.98± 21.90	4	22.21± 8.15	19.90± 7.20	4
96	16.84± 12.73	15.33± 11.75	4	2.83± 0.56	2.47± 0.47	4
120	9.71± 7.22	8.60± 6.33	4	1.71± 0.41	1.48± 0.34	4
144	6.42± 4.23	5.89± 3.95	4	1.10± 0.24	1.00± 0.22	4
168	4.51± 2.92	3.99± 2.60	4	0.78± 0.15	0.71± 0.13	4

Table 11. HPLC/RAD Analysis Conditions for Qualitative Metabolite Profiling of Feces and Urine

Instrument:	Hewlett Packard 1050 High Performance Liquid Chromatograph/Packard Radiomatic FLO-ONE Detector
Column:	Alltima C-18, 5 µm, 250 x 4.6 mm
Mobile Phase for Feces 24 -72hr	A: Water B: 50:50 Acetonitrile:Tetrahydrofuran
Mobile Phase for Feces 6 -12hr & Urine:	A: Water B: Acetonitrile
Gradient:	Time (min) %A %B 0 100 0 20 100 0 40 0 100 60 0 100 65 100 0 80 100 0
Injection:	100 µL injection
Flow Rate:	HPLC at 1.0 mL/min Radiomatic at 3.0 mL/min
Liquid Scintillation Cocktail:	Ultima Flo M

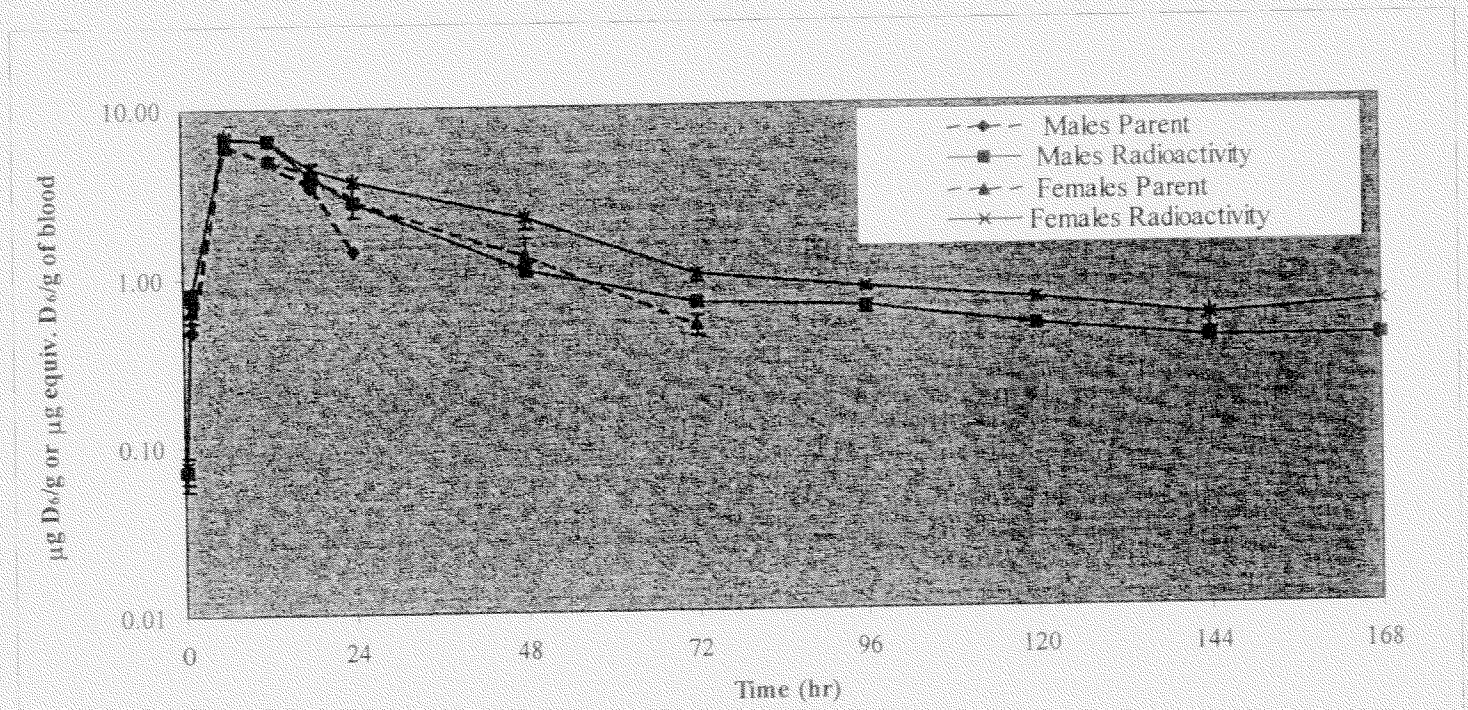
Table 12. Summary of Fecal Metabolites as Percentages of the Total Fecal Radioactivity Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

Sex and Timepoint	% Unknown Major Metabolite	% of Parent D_6
6 Hour Male	NA	NA
6 Hour Female	0%	100%
12 Hour Male	0%± 0%	100%± 0%
12 Hour Female	0%± 0%	100%± 0%
24 Hour Male	1%± 0%	99%± 1%
24 Hour Female	1%± 0%	99%± 1%
48 Hour Male	1%± 0%	99%± 1%
48 Hour Female	2%± 2%	98%± 3%
72 Hour Male	0%± 0%	100%± 0%
72 Hour Female	0%± 0%	100%± 0%

Table 13. Summary of Urinary Metabolites as Percentages of the Total Urinary Radioactivity Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

Sex and Timepoint	De-Methylated % of Methylsilanetriol	De-Methylated % of Dimethyl-disiloxane-1,3,3,3-tetrol	% of Dimethyl-silanediol	Sum of De-methylated Peak Percentages
12 Hour Male	71%± 19%	0%± 0%	29%± 19%	71%± 19%
12 Hour Female	51%± 50%	0%± 0%	49%± 50%	51%± 50%
24 Hour Male	55%± 4%	9%± 6%	36%± 3%	64%± 3%
24 Hour Female	48%± 6%	5%± 9%	47%± 7%	53%± 7%
48 Hour Male	59%± 3%	2%± 4%	39%± 4%	61%± 4%
48 Hour Female	50%± 3%	5%± 9%	45%± 9%	55%± 9%

Figure 1. Blood Radioactivity and Parent D_6 Concentration *vs.* Time in Male and Female Fischer Rats Following Single Oral Administration of ^{14}C-D_6 in Corn Oil



Areas Under The Blood Curves
(µg ^{14}C-Equivalents D_6 X hr/g or µg D_6 X hr/g)

Males
Radioactivity = 225.93 ± 5.75
D_6 Parent = 108.36 ± 3.81

Females
Radioactivity = 293.53 ± 11.32
D_6 Parent = 177.20 ± 12.67

Data expressed as mean ± standard error of the mean

Figure 2. Disposition of Radioactivity in Female and Male Fischer 344 Rats Following Single Oral Dose of ^{14}C-D_6 in Corn Oil

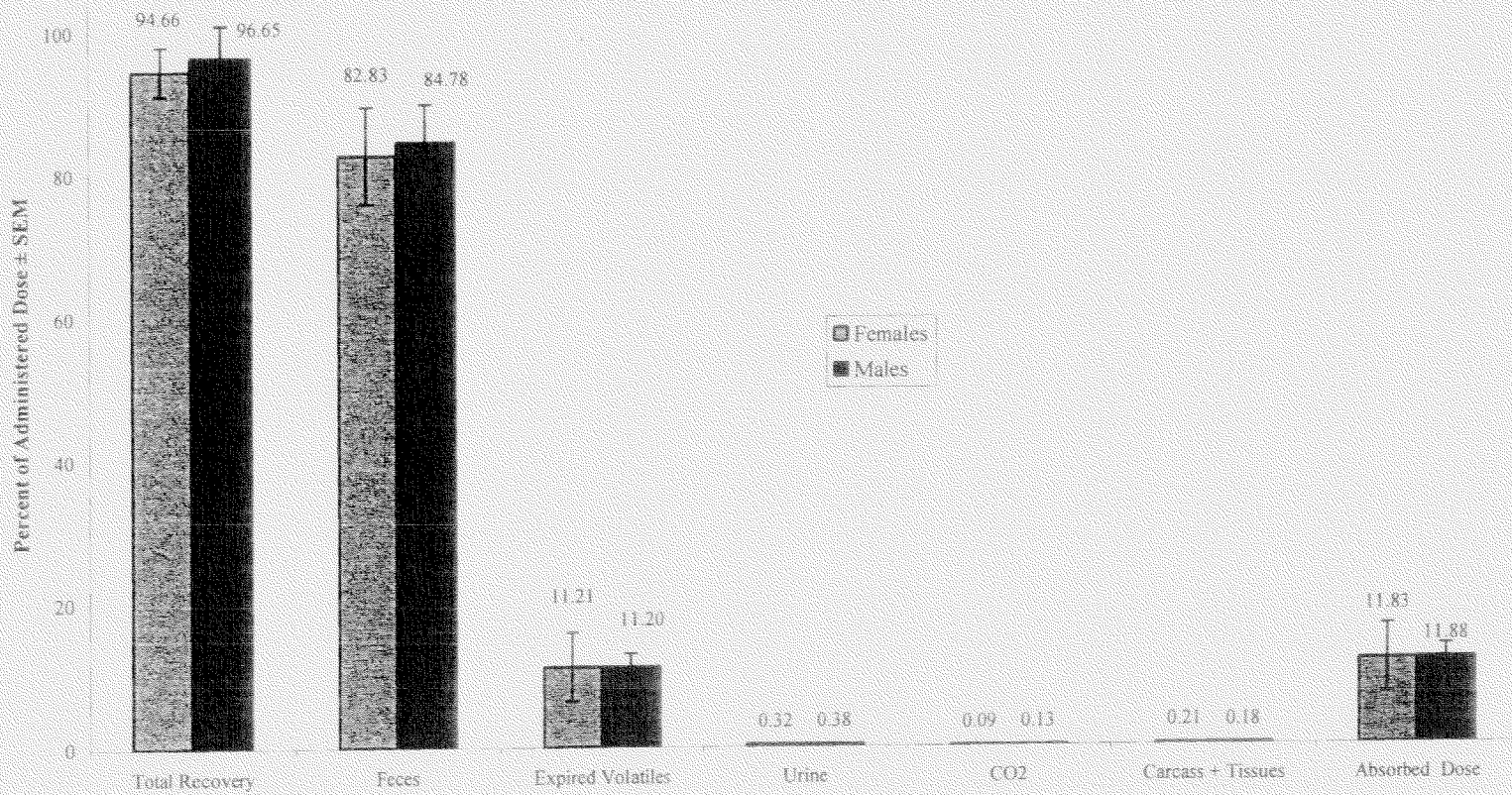
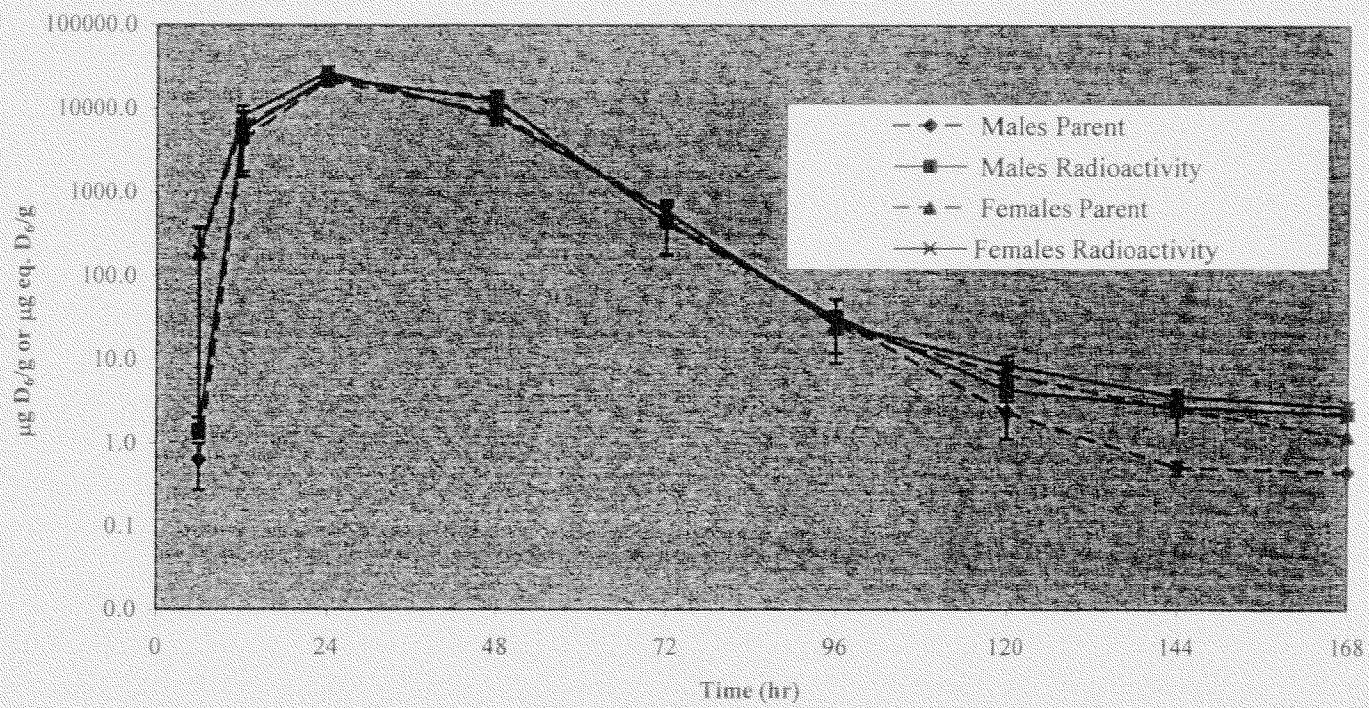


Figure 3. Radioactivity *vs.* Parent D_6 Concentration in Feces of Male and Female Fischer Rats Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

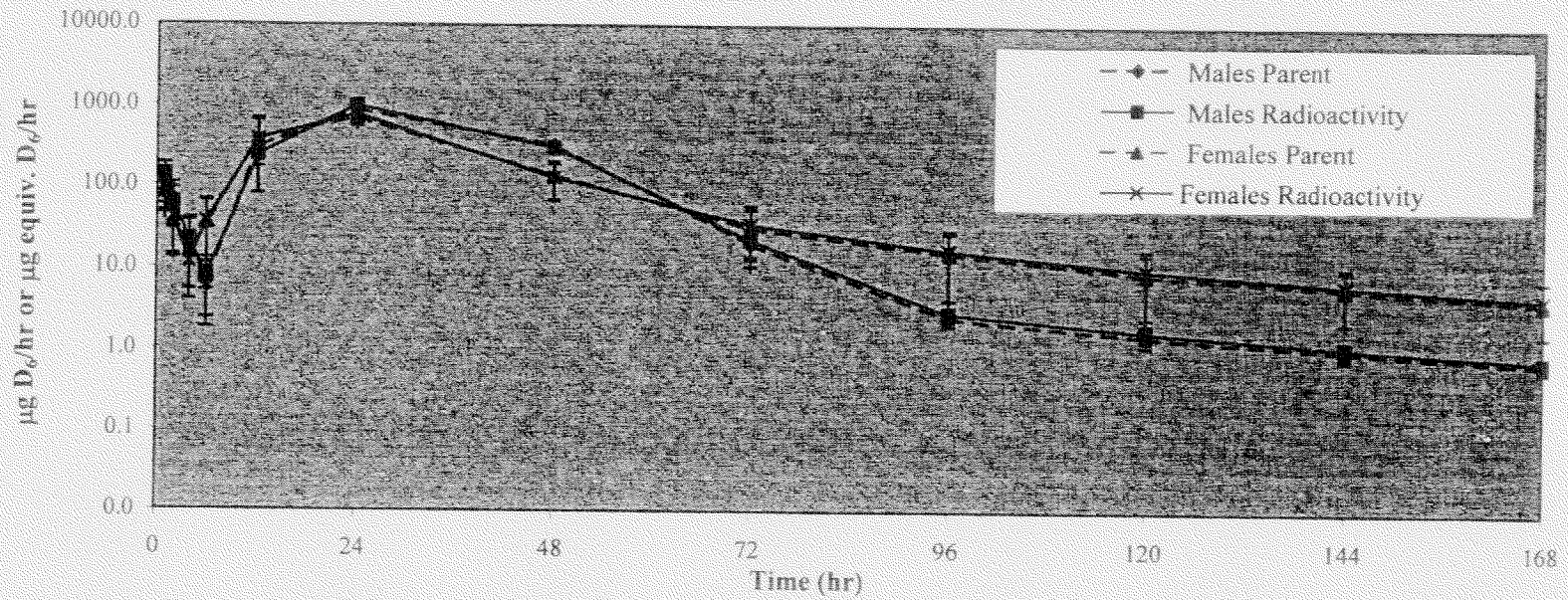


Areas Under The Fecal Curves
(µg ^{14}C-Equivalents D_6 X hr/g or µg D_6 X hr/g)

Males	Females
Radioactivity = 696291.26 ± 58467.71	Radioactivity = 664925.90 ± 73399.84
D_6 Parent = 695573.24 ± 74837.96	D_6 Parent = 596859.84 ± 44845.95

Data expressed as mean ± standard error of the mean

Figure 4. Radioactivity *vs.* Parent D_6 Content in Charcoal Tubes Used to Trap Expired Volatiles in Male and Female Fischer Rats Following Single, Oral Administration of ^{14}C-D_6 in Corn Oil



Areas Under The Expired Volatile Curves
(µg ^{14}C-Equivalents D_6 X hr/g or µg D_6 X hr/g)

Males	Females
Radioactivity = 24730.65 ± 3116.15	Radioactivity = 19101.98 ± 4128.90
D_6 Parent = 23759.53 ± 2673.09	D_6 Parent = 17887.77 ± 3802.00

Data expressed as mean ± standard error of the mean

Figure 5. Example HPLC/RAD Chromatograms of a) ^{14}C-D_6 Standard and Urinary Profiles Obtained from b) Male and c) Female Fischer 344 Rats 24 Hours After Single Oral Administration of ^{14}C-D_6 in Corn Oil

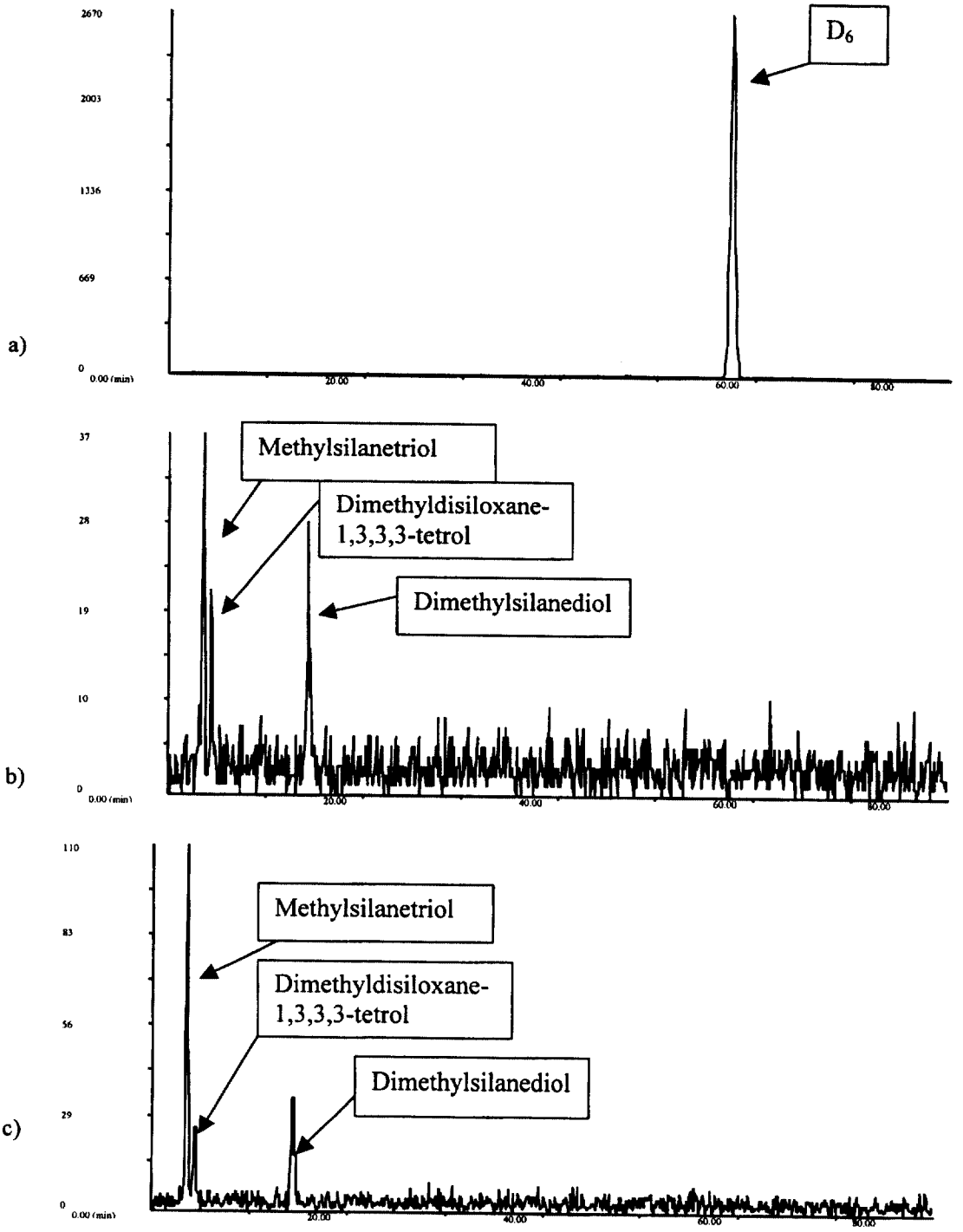
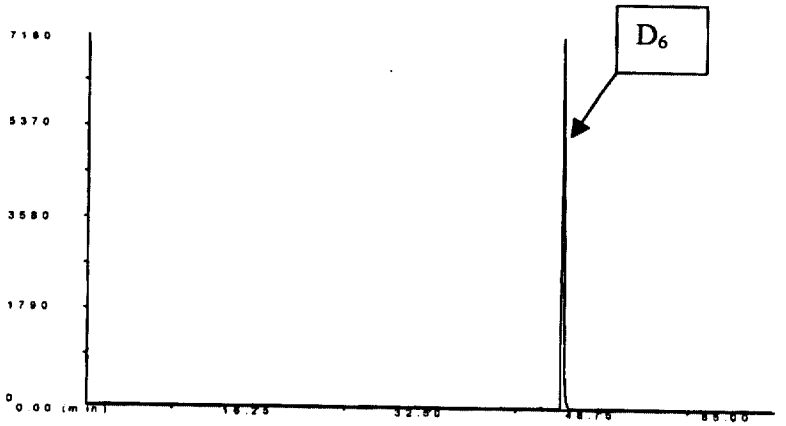
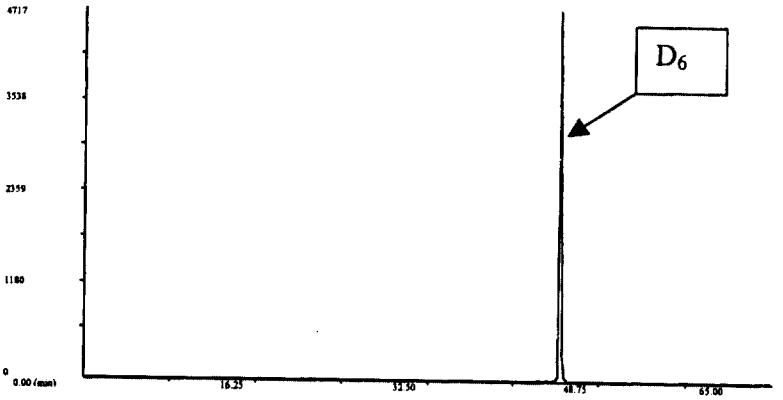


Figure 6. Example HPLC/RAD Chromatograms of a) ^{14}C-D_6 Standard and Fecal Profiles Obtained from b) Male and c) Female Fischer 344 Rats 24 Hours After Single Oral Administration of ^{14}C-D_6 in Corn Oil

a)



b)



c)

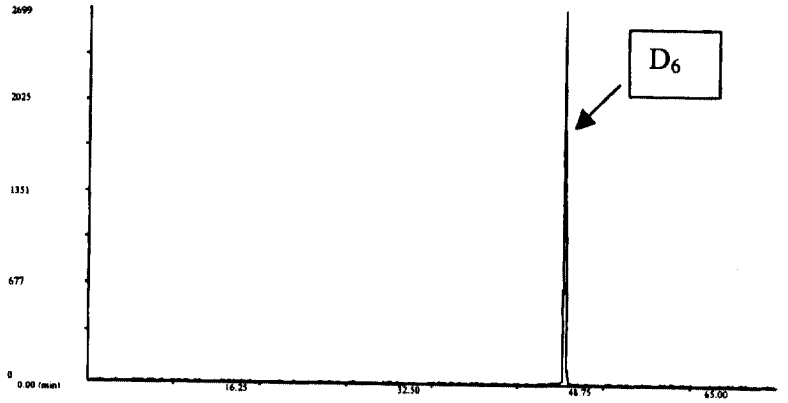


Figure 7. Radioactivity Content in Expired Volatiles and Fecal Material Over Time in Female Rats Following Single Oral Administration of ^{14}C- D_6 in Corn Oil

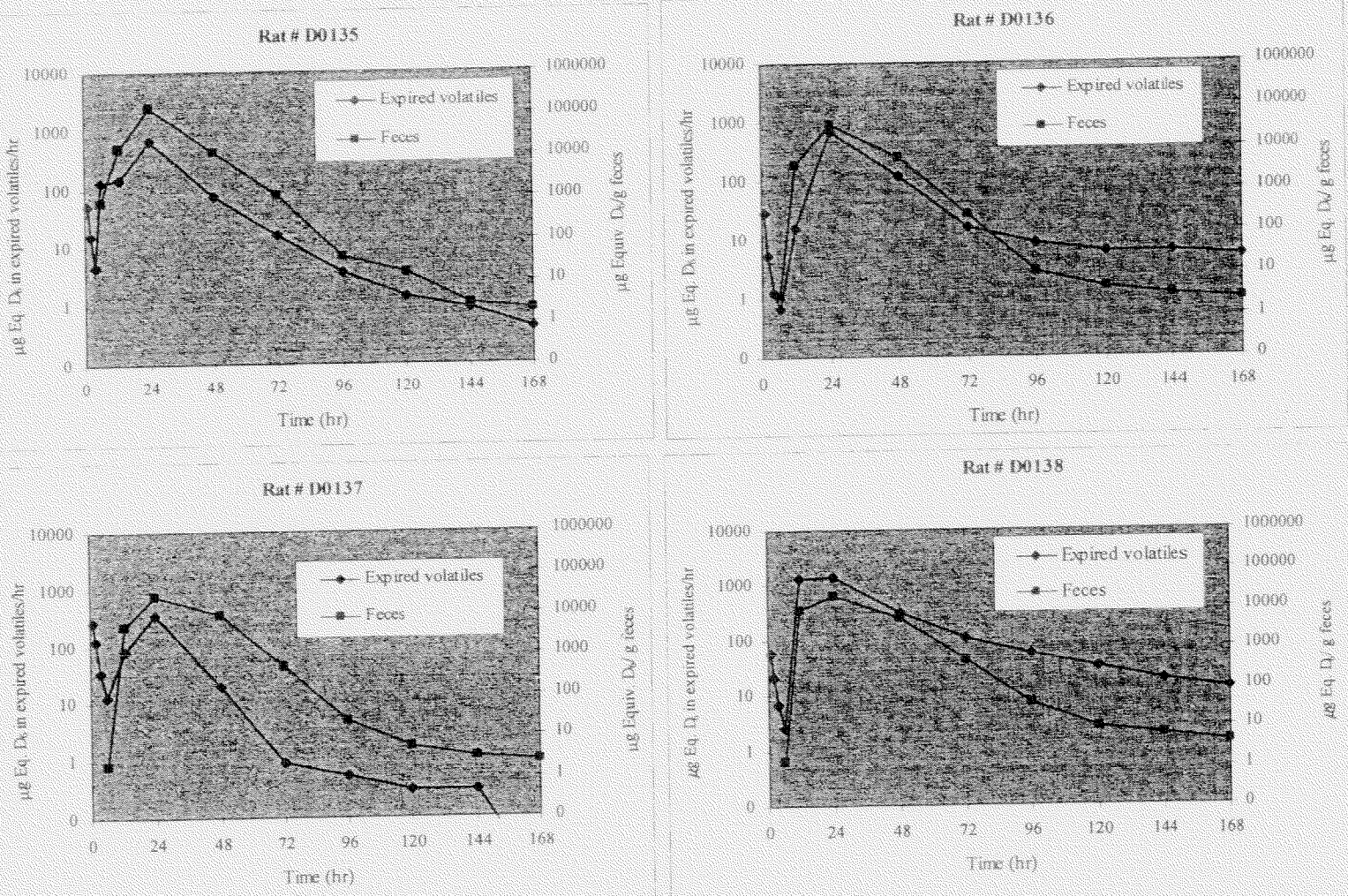


Figure 8. Radioactivity Content in Expired Volatiles and Fecal Material Over Time in Male Rats Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

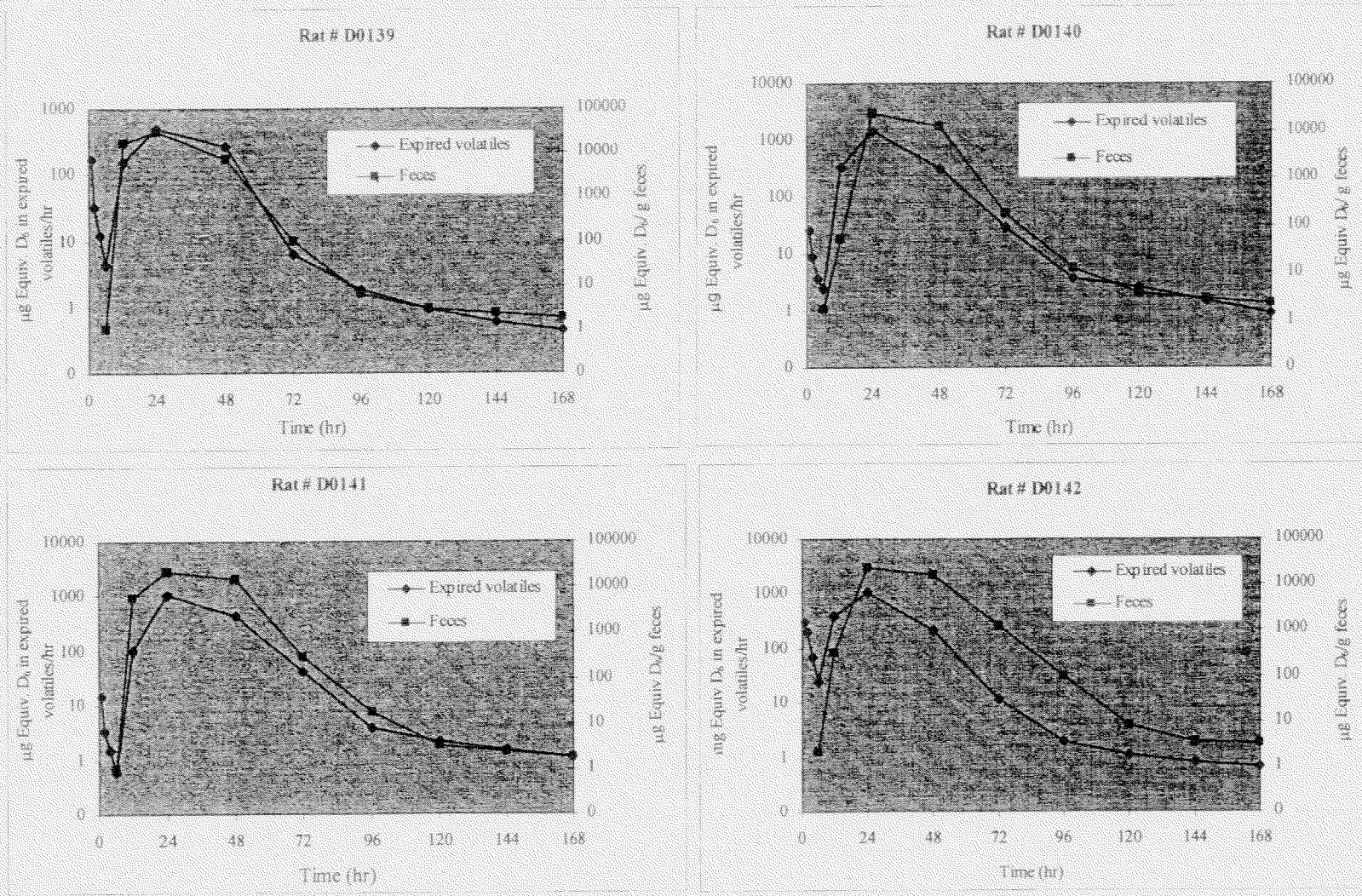


Figure 9. Whole-Body Autoradiography of Female Fischer 344 Rats 1, 4, 12, 24, 48, 96 and 168 Hours Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

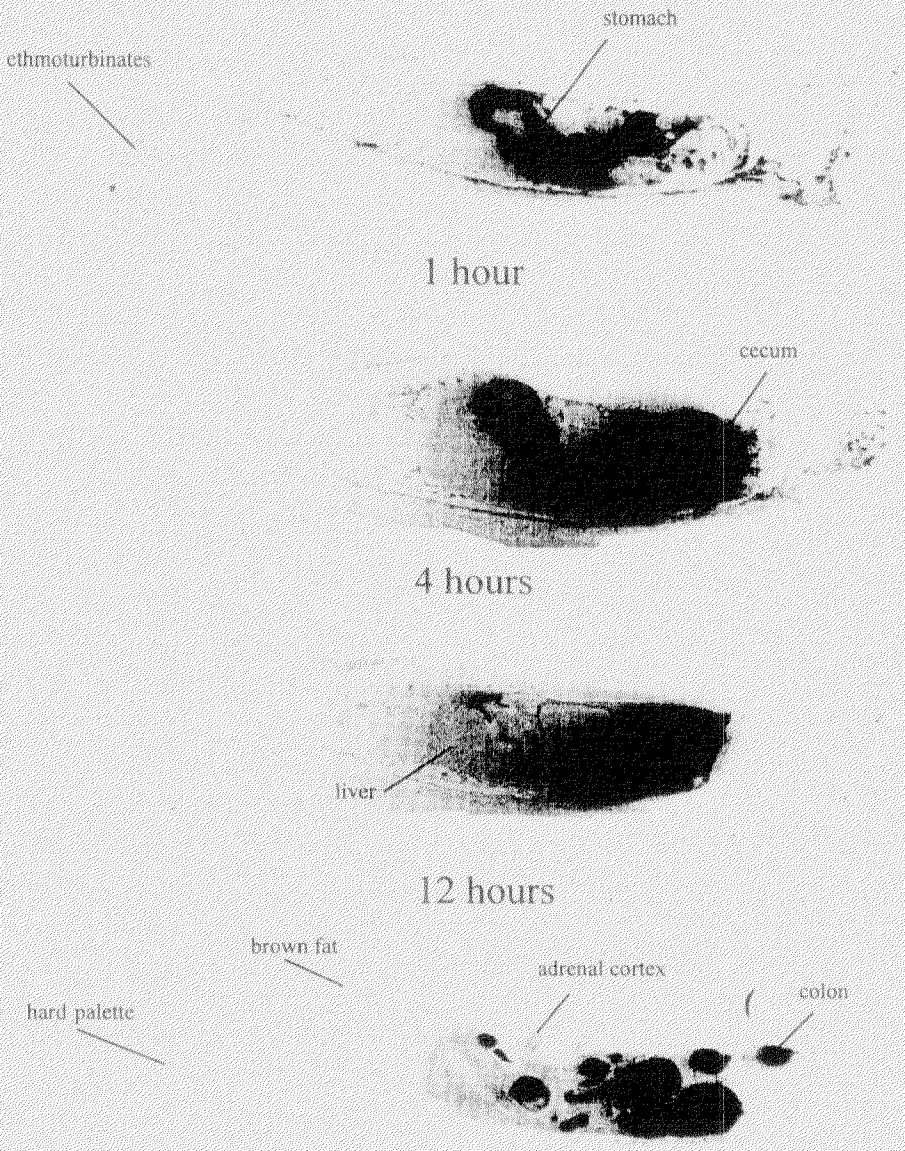
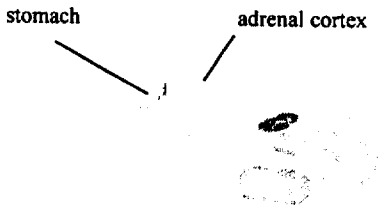
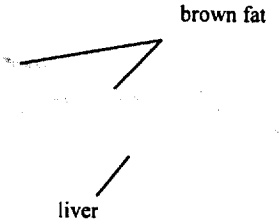


Figure 9 (continued). Whole-Body Autoradiography of Female Fischer 344 Rats 1, 4, 12, 24, 48, 96 and 168 Hours Following Single Oral Administration of ^{14}C-D_6 in Corn Oil



48 hours

96 hours



168 hours

Figure 10. Whole-Body Autoradiography of Male Fischer 344 rats 1, 4, 12, 24, 48, 96 and 168 Hours Following Single Oral Administration of ^{14}C-D_6 in Corn Oil

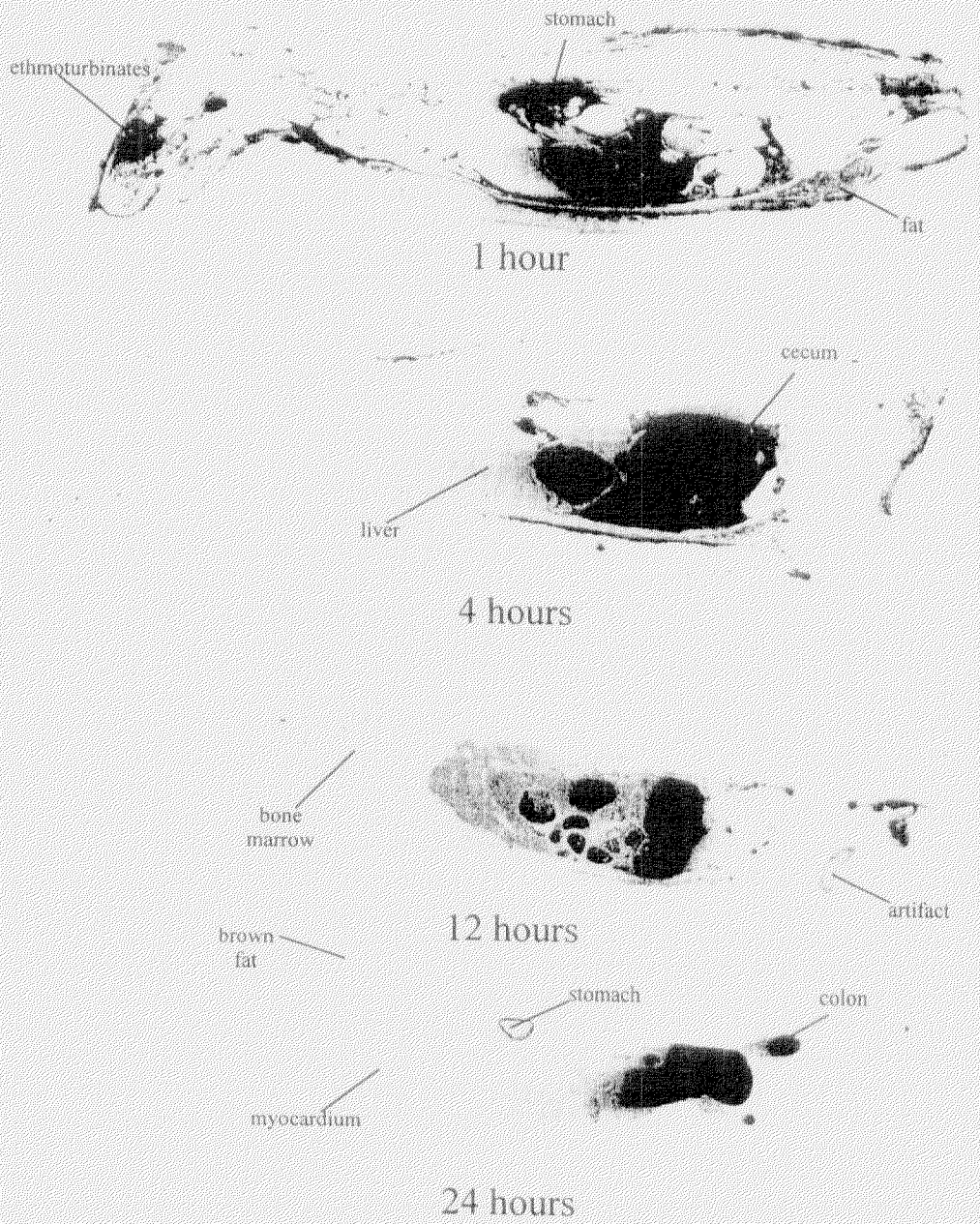
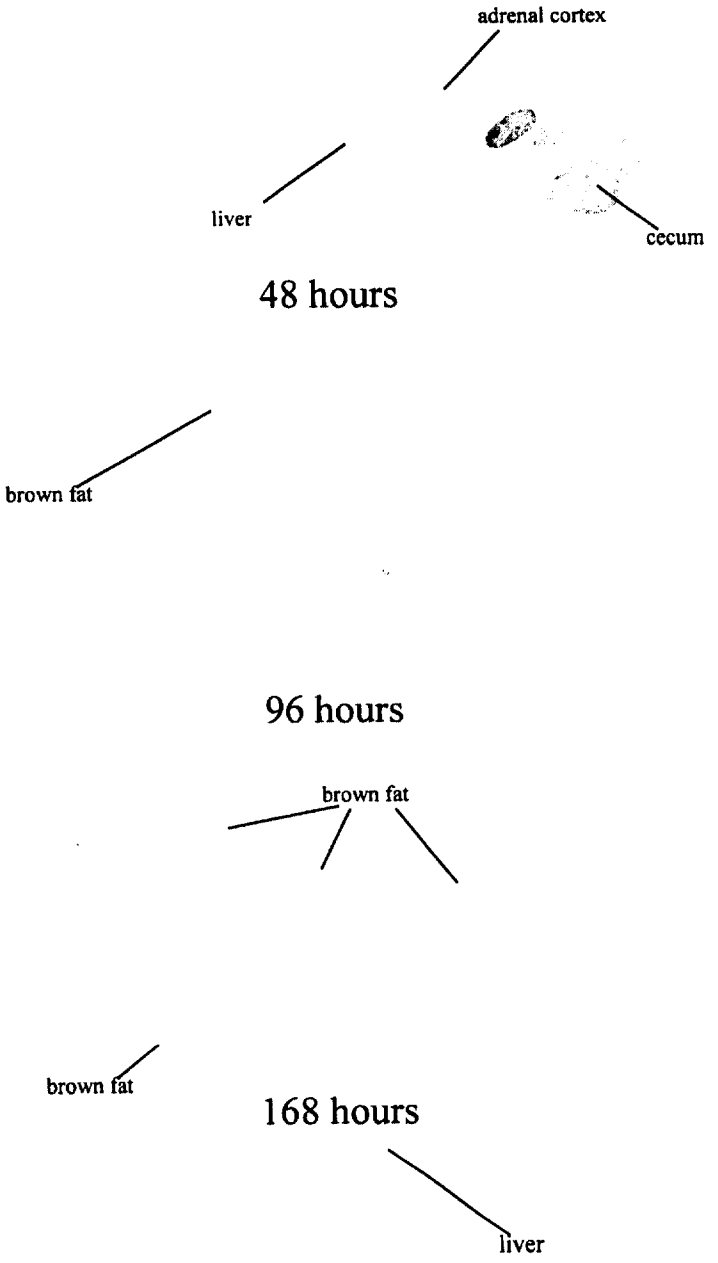


Figure 10 (continued). Whole-Body Autoradiography of Male Fischer 344 rats 1, 4, 12, 24, 48, 96 and 168 Hours Following Single Oral Administration of ^{14}C-D_6 in Corn Oil



Appendix A

Parent Quantitation of D_6 in Blood, Feces and Expired Volatiles, and Radioactivity Spreadsheets

Calculations and Abbreviations

Calculations

1. **Dosing Solution**

Targeted dose : 1g D_6/ 1000 g BW

D_6 in carrier: Calculated dose (g) = $\frac{\text{Body Weight (g)/1000}}{\text{D}_6 \text{ concentration (mg D}_6\text{/g d.s.)/1000mg/g}}$

2. **Radioactivity**
   1 μCi = 2.22 x 10^6 DPMs

3. **Specific activity of dosing solution**
   DPM/mg of dosing solution / 2.22 x 10^6 (DPM/μCi) = μCi/mg of dosing solution

4. **D_6 Specific activity (DPM/ mg D_6)**
   DPM/mg of dosing solution X 1000 (mg/g)/D_6 concentration (mg D_6)/ g of dosing solution

5. **Dose (DPMs)**
   S.A. of dosing solution (DPMs/mg) X dose (mg) = DPMs

6. **Dose (μCi)**
   S.A. of dosing solution (μCi/mg) X dose (mg) = μCi

7. **Matrix Background**
   Average DPM/g of control samples

8. **Radioactivity concentration in the sample aliquots (Corrected DPM/g)**
   Aliquot DPM/Aliquot Wt.(g) - matrix background (DPM/g)

9. **Total DPMs in the sample**
   Radioactivity conc. (DPM/g) X Sample wt. [a] (g) = DPM

   [a]sample wt. = homogenate wt.(e.g. feces), extract wt. (e.g. charcoal) or direct sample wt. (e.g. urine)

10. **Percent of administered dose**
    $\frac{\text{Total DPMs in sample}}{\text{Dose (DPMs)}}$ X 100 = % Dose

11. **Average percent dose**
    Percent dose for the group of test systems

12. **Cumulative percent dose**
    Percent of administered dose collected in excreta added to the percent of the applied dose of the previous timepoint to get the total percent recovery in urine, feces, KOH and charcoal tubes over duration of exposure

13. Percent dose eliminated through GI Tract
Percent of administered dose excreted in feces over 168 hour

14. Correction for solubilization processing efficiency
Percent of administered dose recovered in feces x 100/ processing efficiency

15. Percent of the total recovery
Percent of administered dose recovered in the sample (e.g. excreta) x 100/Total % dose recovered in all the samples.

16. Equivalent μg D_6/g of specimen

$$\frac{\text{Total DPM in the sample}^a \text{ / specimen}^b \text{ weight (g) X 1000 μg/mg}}{\text{Specific activity of } D_6 \text{ in dosing solution (DPM/mg } D_6)}$$

[a]Sample = homogenate (e.g. feces), extract (e.g. charcoal) or direct sample (e.g. urine, KOH)
[b]Specimen = tissue, extract (charcoal), or excreta (e.g. feces, urine)

17. Equivalent μg D_6 in expired volatiles/hr

$$\frac{\text{Total DPM in the sample}^a \text{ X 1000 (μg/mg) / time interval (hr)}}{\text{Specific activity of } D_6 \text{ in dosing solution (DPM/mg } D_6)}$$

Time interval (hr) = Time between charcoal tube collections = Time of charcoal tube in use
[a]Sample = Toluene extracts (charcoal)

18. Reanalysis Criteria (%RR)
The %RR is derived by calculating the difference between two values (DPM/g) and expressing this difference as a percentage of the mean for the two values (aliquots). Samples which fail to meet the following criteria were re-analyzed in duplicate:

Note: F1, F2, and F3 are flags on Excel data sheets; the flags are used if criteria defined below are not met

A) For DPM/g between 0 and 299 the %RR must be less than or equal to 20% (F1)
B) For DPM/g between 300 and 999 the %RR must be less than or equal to 15% (F2)
C) For DPM/g greater than 1000, the %RR must be less than 10%(F3)

An exception to the above criteria is when the % dose recovered in the sample is less than or equal to 0.1%, than no re-analysis of a sample is required.

19. BLQ
Values below limit of quantification (BLQ) are considered to be equal to zero.

20. LOQ expressed as μg D_6/g (blood, feces)
LOQ in extract / average sample weight

21. LOQ expressed as μg D_6/hr (expired volatiles)
LOQ in extract x average sample weight / length of time tube used (24 hours)

22. Parent μg D_6/hr (expired volatiles)

μg D_6/g X sample[a] weight (g) / length of time tube used (hr)

[a]Sample = Toluene extracts (charcoal)

Abbreviations

DPM = Disintegrations per minute	B.W. = Body weight
Avg. = Average	N.D. = Not determined
Sol'n. = Solution	LOQ = Limit of quantification
S.A.= Specific Activity	BLQ = Below limit of quantification
d.s. = Dosing solution	ALQ= Above limit of quantification
Cum. = Cumulative	IE = Injection Error
Conc. = Concentration	nd = Not Detected
Rep. = Replicate	Hr. = Hour
WBA = Whole Body Autoradiography	F = Flag indicating non-uniformity of the sampling
MB = Mass Balance	Homog., Homogen. = Homogenate
BK = Blood Kinetics	CO = Corn Oil
GI tract = Gastrointestinal Tract	%RR = Percent Relative Range
Wt. = Weight	THF = Tetrahydrofuran
St.Dev. = SD = Standard Deviation	TEAH = Tetraethylammonium hydroxide
Eq./Equiv. = Equivalent	GC = Gas chromatography
SEM = Standard error of the mean	MS = MSD = Mass Spectrometry
Bkg. = Background	HPLC = High performance liquid chromatography
N.S. = No sample	LSC = Liquid Scintillation Counter
N.A. or N/AP= Not applicable	

Blood

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 uCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

Blood

Limit of Quantitation was determined to be *0.45* and was used in place of BLQ for groups/timepoints that contained at least one sample above BLQ

Animal ID	Time Point	Group ID / Sex	Parent µg D_6/g	Parent µg D_6/g of blood Avg./Sex/ Timepoint	SEM	Radioactivity µg Eq. D_6/g blood	Radioactivity µg Eq. D_6/g of blood/ Avg./ Sex/ Timepoint	SEM
D0157	24h	7/Female	NA			NA		
D0158	24h	7/Female	NA			NA		
D0159	24h	8/Male	NA			NA		
D0160	24h	8/Male	Sample Excluded From Study					
D0161	15min	9/Female	BLQ	BLQ	NA	0.07	0.08	0.015
D0162	15min	9/Female	BLQ			0.07		
D0163	15min	9/Female	BLQ			0.14		
D0164	15min	9/Female	BLQ			0.09		
D0165	15min	9/Female	BLQ			0.07		
D0166	15min	9/Female	BLQ			0.03		
D0167	15min	10/Male	BLQ	BLQ	NA	0.08	0.07	0.017
D0168	15min	10/Male	BLQ			0.13		
D0169	15min	10/Male	BLQ			0.04		
D0170	15min	10/Male	BLQ			0.10		
D0171	15min	10/Male	BLQ			0.01		
D0172	15min	10/Male	BLQ			0.07		
D0161	60min	9/Female	1.02	0.64	0.086	1.20	0.79	0.096
D0162	60min	9/Female	0.68			0.76		
D0163	60min	9/Female	0.62			0.73		
D0164	60min	9/Female	*0.45*			0.71		
D0165	60min	9/Female	0.62			0.86		
D0166	60min	9/Female	*0.45*			0.48		
D0167	60min	10/Male	*0.45*	0.49	0.027	0.65	0.81	0.053
D0168	60min	10/Male	*0.45*			0.97		
D0169	60min	10/Male	0.61			0.66		
D0170	60min	10/Male	*0.45*			0.82		
D0171	60min	10/Male	0.53			0.91		
D0172	60min	10/Male	*0.45*			0.85		
D0161	6h	9/Female	8.20	6.10	0.551	10.81	6.80	0.901
D0162	6h	9/Female	5.79			7.43		
D0163	6h	9/Female	5.03			5.76		
D0164	6h	9/Female	4.38			4.83		

Rounding differences may occur due to electronic handling of numbers

Blood

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 uCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

Blood

Limit of Quantitation was determined to be *0.45* and was used in place of BLQ for groups/timepoints that contained at least one sample above BLQ

Animal ID	Time Point	Group ID / Sex	Parent µg D_6/g	Parent µg D_6/g of blood Avg./Sex/ Timepoint	SEM	Radioactivity µg Eq. D_6/g blood	Radioactivity µg Eq. D_6/g of blood/ Avg./ Sex/ Timepoint	SEM
D0165	6h	9/Female	6.54			5.10		
D0166	6h	9/Female	6.66			6.84		
D0167	6h	10/Male	7.35	6.38	0.345	7.89	6.60	0.630
D0168	6h	10/Male	6.30			5.44		
D0169	6h	10/Male	6.04			5.47		
D0170	6h	10/Male	5.41			5.22		
D0171	6h	10/Male	5.72			6.58		
D0172	6h	10/Male	7.44			9.00		
D0161	12h	9/Female	6.40	4.96	0.325	7.67	6.41	0.438
D0162	12h	9/Female	4.94			6.32		
D0163	12h	9/Female	4.75			5.94		
D0164	12h	9/Female	5.11			7.76		
D0165	12h	9/Female	4.53			5.53		
D0166	12h	9/Female	4.05			5.25		
D0167	12h	10/Male	5.64	4.97	0.395	8.20	6.49	0.383
D0168	12h	10/Male	3.85			5.86		
D0169	12h	10/Male	3.66			6.02		
D0170	12h	10/Male	5.96			6.05		
D0171	12h	10/Male	5.32			5.82		
D0172	12h	10/Male	5.37			6.97		
D0161	18h	9/Female	6.64	4.15	0.570	6.55	4.42	0.482
D0162	18h	9/Female	4.48			4.58		
D0163	18h	9/Female	3.35			3.35		
D0164	18h	9/Female	4.45			4.67		
D0165	18h	9/Female	2.89			3.89		
D0166	18h	9/Female	3.08			3.46		
D0167	18h	10/Male	4.05	3.45	0.201	4.84	3.97	0.261
D0168	18h	10/Male	2.92			3.95		
D0169	18h	10/Male	3.02			3.15		
D0170	18h	10/Male	3.54			3.39		
D0171	18h	10/Male	3.19			4.48		
D0172	18h	10/Male	4.01			3.98		

Rounding differences may occur due to electronic handling of numbers

Blood

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 uCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

Blood

Limit of Quantitation was determined to be *0.45* and was used in place of BLQ for groups/timepoints that contained at least one sample above BLQ

Animal ID	Time Point	Group ID / Sex	Parent µg D_6/g	Parent µg D_6/g of blood Avg./Sex/ Timepoint	SEM	Radioactivity µg Eq. D_6/g blood	Radioactivity µg Eq. D_6/g of blood/ Avg./ Sex/ Timepoint	SEM
D0161	24h	9/Female	5.62	2.87	0.578	4.87	3.73	0.305
D0162	24h	9/Female	2.54			3.79		
D0163	24h	9/Female	2.04			3.25		
D0164	24h	9/Female	2.95			4.28		
D0165	24h	9/Female	2.42			3.33		
D0166	24h	9/Female	1.66			2.84		
D0167	24h	10/Male	1.61	1.42	0.130	2.99	2.78	0.141
D0168	24h	10/Male	1.76			2.70		
D0169	24h	10/Male	0.94			2.56		
D0170	24h	10/Male	1.17			2.26		
D0171	24h	10/Male	1.39			2.97		
D0172	24h	10/Male	1.67			3.22		
D0161	48h	9/Female	1.26	1.34	0.317	2.17	2.22	0.309
D0162	48h	9/Female	1.14			2.28		
D0163	48h	9/Female	1.32			2.31		
D0164	48h	9/Female	0.97			1.80		
D0165	48h	9/Female	2.81			3.52		
D0166	48h	9/Female	0.53			1.23		
D0167	48h	10/Male	BLQ	BLQ	NA	1.08	1.09	0.096
D0168	48h	10/Male	BLQ			1.00		
D0169	48h	10/Male	BLQ			1.54		
D0170	48h	10/Male	BLQ			0.96		
D0171	48h	10/Male	BLQ			0.87		
D0172	48h	10/Male	BLQ			1.09		
D0161	72h	9/Female	NS	0.50	0.072	NS	0.99	0.092
D0162	72h	9/Female	0.39			0.92		
D0163	72h	9/Female	NS			NS		
D0164	72h	9/Female	*0.45*			0.97		
D0165	72h	9/Female	0.71			1.25		
D0166	72h	9/Female	*0.45*			0.82		
D0167	72h	10/Male	BLQ	BLQ	NA	0.59	0.68	0.037
D0168	72h	10/Male	BLQ			0.71		

Rounding differences may occur due to electronic handling of numbers

Blood

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 uCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

Blood

Limit of Quantitation was determined to be *0.45* and was used in place of BLQ for groups/timepoints that contained at least one sample above BLQ

Animal ID	Time Point	Group ID / Sex	Parent µg D_6/g	Parent µg D_6/g of blood Avg./Sex/ Timepoint	SEM	Radioactivity µg Eq. D_6/g blood	Radioactivity µg Eq. D_6/g of blood/ Avg./ Sex/ Timepoint	SEM
D0169	72h	10/Male	BLQ			0.69		
D0170	72h	10/Male	BLQ			0.57		
D0171	72h	10/Male	BLQ			0.67		
D0172	72h	10/Male	BLQ			0.83		
D0161	96h	9/Female	NS	BLQ	NA	NS	0.82	0.048
D0162	96h	9/Female	BLQ			0.85		
D0163	96h	9/Female	NS			NS		
D0164	96h	9/Female	BLQ			0.88		
D0165	96h	9/Female	BLQ			0.86		
D0166	96h	9/Female	BLQ			0.67		
D0167	96h	10/Male	BLQ	BLQ	NA	0.52	0.63	0.052
D0168	96h	10/Male	BLQ			0.80		
D0169	96h	10/Male	BLQ			0.66		
D0170	96h	10/Male	BLQ			0.52		
D0171	96h	10/Male	BLQ			0.64		
D0172	96h	10/Male	NS			NS		
D0161	120h	9/Female	NS	BLQ	NA	NS	0.69	0.044
D0162	120h	9/Female	BLQ			0.72		
D0163	120h	9/Female	NS			NS		
D0164	120h	9/Female	BLQ			0.74		
D0165	120h	9/Female	BLQ			0.60		
D0166	120h	9/Female	NS			NS		
D0167	120h	10/Male	BLQ	BLQ	NA	0.48	0.47	0.010
D0168	120h	10/Male	BLQ			0.43		
D0169	120h	10/Male	BLQ			0.48		
D0170	120h	10/Male	BLQ			0.47		
D0171	120h	10/Male	BLQ			0.49		
D0172	120h	10/Male	NS			NS		
D0161	144h	9/Female	NS	BLQ	NA	NS	0.52	0.092
D0162	144h	9/Female	NS			NS		

Rounding differences may occur due to electronic handling of numbers

Blood

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 uCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

Blood

Limit of Quantitation was determined to be *0.45* and was used in place of BLQ for groups/timepoints that contained at least one sample above BLQ

Animal ID	Time Point	Group ID / Sex	Parent µg D_6/g	Parent µg D_6/g of blood Avg./Sex/ Timepoint	SEM	Radioactivity µg Eq. D_6/g blood	Radioactivity µg Eq. D_6/g of blood/ Avg./ Sex/ Timepoint	SEM
D0163	144h	9/Female	NS			NS		
D0164	144h	9/Female	BLQ			0.61		
D0165	144h	9/Female	BLQ			0.43		
D0166	144h	9/Female	NS			NS		
D0167	144h	10/Male	NS	BLQ	NA	NS	0.39	0.037
D0168	144h	10/Male	BLQ			0.38		
D0169	144h	10/Male	BLQ			0.33		
D0170	144h	10/Male	BLQ			0.46		
D0171	144h	10/Male	NS			NS		
D0172	144h	10/Male	NS			NS		
D0161	168h	9/Female	NS	BLQ	NA	NS	0.61	0.042
D0162	168h	9/Female	BLQ			0.54		
D0163	168h	9/Female	BLQ			0.72		
D0164	168h	9/Female	NS			NS		
D0165	168h	9/Female	BLQ			0.63		
D0166	168h	9/Female	BLQ			0.55		
D0167	168h	10/Male	BLQ	BLQ	NA	0.37	0.39	0.037
D0168	168h	10/Male	BLQ			0.30		
D0169	168h	10/Male	BLQ			0.28		
D0170	168h	10/Male	BLQ			0.46		
D0171	168h	10/Male	BLQ			0.52		
D0172	168h	10/Male	BLQ			0.39		

Rounding differences may occur due to electronic handling of numbers

Feces

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 µCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

Feces

Animal ID	Time Point	Group ID/ Sex	Radioactivity Conc. µg Eq D_6/g of Feces	Avg. Conc. µg Eq D_6/g of Feces	SD	SEM	Parent µg D_6/g feces	Parent µg D_6/g Avg./ sex/ timepoint	SD	SEM	% Dose Recovered in Feces	Cum. % Dose Recovered in Feces	Average Cum. % Dose Recovered per Group	SD	SEM
D0135	6hr	3/F	773.77				737.50				0.843	0.843			
D0136	6hr	3/F	3.03				2.86				0.003	0.003			
D0137	6hr	3/F	1.82				1.60				0.001	0.001			
D0138	6hr	3/F	1.31	194.98	385.856	192.928	1.03	185.75	367.838	183.919	0.001	0.001	0.212	0.420	0.210
D0139	6hr	4/M	0.95				0.38				0.000	0.000			
D0140	6hr	4/M	1.66				0.48				0.000	0.000			
D0141	6hr	4/M	1.01				BLQ				0.001	0.001			
D0142	6hr	4/M	2.12	1.44	0.556	0.278	1.69	0.85	0.726	0.363	0.001	0.001	0.001	0.000	0.000
D0135	12hr	3/F	15025.32				8007.29				3.640	4.483			
D0136	12hr	3/F	3841.59				3481.94				3.198	3.201			
D0137	12hr	3/F	4908.05				4943.54				5.482	5.484			
D0138	12hr	3/F	8925.91	8175.22	5064.324	2532.162	8529.52	6240.57	2425.759	1212.879	8.758	8.759	5.482	2.376	1.188
D0139	12hr	4/M	15525.79				10683.84				12.777	12.777			
D0140	12hr	4/M	49.24				47.48				0.012	0.012			
D0141	12hr	4/M	5667.57				5278.53				2.647	2.648			
D0142	12hr	4/M	314.65	5389.31	7236.351	3618.175	297.68	4076.88	5020.435	2510.218	0.134	0.135	3.893	6.046	3.023
D0135	24hr	3/F	73948.52				71309.24				28.691	33.174			
D0136	24hr	3/F	35182.83				21223.32				60.398	63.600			
D0137	24hr	3/F	25281.07				21163.30				48.514	53.998			
D0138	24hr	3/F	21407.90	27290.60	7103.930	4101.456	23356.59	21914.40	1249.332	721.302	35.938	44.697	48.867	13.001	6.500
D0139	24hr	4/M	28590.80				21528.03				56.875	69.653			
D0140	24hr	4/M	22102.07				25628.91				25.847	25.859			
D0141	24hr	4/M	21012.38				19701.29				29.307	31.955			
D0142	24hr	4/M	22847.70	23638.24	3386.628	1693.314	23153.22	22502.86	2516.234	1258.117	30.651	30.786	39.563	20.233	10.116
D0135	48hr	3/F	11926.34				13985.39				31.552	64.725			
D0136	48hr	3/F	5508.00				6171.37				16.266	79.866			
D0137	48hr	3/F	8829.52				8899.24				26.525	80.524			
D0138	48hr	3/F	5580.05	7960.98	3064.007	1532.003	6769.59	8956.40	3551.173	1775.586	10.506	55.203	70.079	12.313	6.156
D0139	48hr	4/M	7061.81				5768.29				16.487	86.139			
D0140	48hr	4/M	11758.82				11874.54				45.637	71.497			
D0141	48hr	4/M	15152.13				17883.48				38.625	70.580			
D0142	48hr	4/M	15665.61	12409.59	3964.234	1982.117	18203.21	13432.38	5880.427	2940.214	31.824	62.610	72.706	9.804	4.902
D0135	72hr	3/F	1101.29				1197.59				3.297	68.022			
D0136	72hr	3/F	238.43				242.39				0.667	80.533			
D0137	72hr	3/F	512.50				503.92				1.672	82.196			
D0138	72hr	3/F	463.02	578.81	368.170	184.085	447.11	597.76	415.366	207.683	1.516	56.719	71.868	11.917	5.958
D0139	72hr	4/M	95.87				91.76				0.277	86.417			
D0140	72hr	4/M	169.29				166.80				0.647	72.144			
D0141	72hr	4/M	286.57				276.33				0.662	71.242			
D0142	72hr	4/M	1210.45	440.54	519.244	259.622	1194.87	432.44	513.906	256.953	2.124	64.734	73.634	9.139	4.569

D0135 24hr data excluded due to possible incorrect feces weight

Rounding differences may occur due to electronic handling of numbers

Feces

Feces

Animal ID	Time Point	Group ID/ Sex	Radioactivity Conc. µg Eq D_6/g of Feces	Avg. Conc. µg Eq D_6/g of Feces	SD	SEM	Parent µg D_6/g feces	Parent µg D_6/g Avg./ sex/ timepoint	SD	SEM	% Dose Recovered in Feces	Cum. % Dose Recovered in Feces	Average Cum. % Dose Recovered per Group	SD	SEM
D0135	96hr	3/F	37.20				35.54				0.135	68.157			
D0136	96hr	3/F	10.15				8.65				0.071	80.604			
D0137	96hr	3/F	23.41				21.67				0.077	82.274			
D0138	96hr	3/F	37.34	27.02	13.012	6.506	35.21	25.26	12.824	6.412	0.124	56.843	71.970	11.889	5.945
D0139	96hr	4/M	6.03				3.36				0.020	86.437			
D0140	96hr	4/M	12.72				10.09				0.046	72.190			
D0141	96hr	4/M	17.59				14.09				0.060	71.301			
D0142	96hr	4/M	92.98	32.33	40.713	20.357	95.12	30.67	43.194	21.597	0.185	64.919	73.712	9.080	4.540
D0135	120hr	3/F	15.23				12.28				0.073	68.230			
D0136	120hr	3/F	4.32				3.28				0.016	80.620			
D0137	120hr	3/F	5.48				2.35				0.019	82.293			
D0138	120hr	3/F	9.34	8.59	4.921	2.461	8.91	6.71	4.715	2.358	0.030	56.873	72.004	11.878	5.939
D0139	120hr	4/M	2.78				0.79				0.010	86.447			
D0140	120hr	4/M	3.66				1.57				0.014	72.204			
D0141	120hr	4/M	3.22				1.12				0.012	71.313			
D0142	120hr	4/M	7.93	4.40	2.381	1.191	6.10	2.40	2.489	1.244	0.023	64.942	73.726	9.076	4.538
D0135	144hr	3/F	2.71				1.11				0.010	68.240			
D0136	144hr	3/F	2.94				1.60				0.011	80.631			
D0137	144hr	3/F	3.09				1.05				0.011	82.304			
D0138	144hr	3/F	6.01	3.69	1.558	0.779	6.50	2.57	2.636	1.318	0.019	56.892	72.017	11.875	5.937
D0139	144hr	4/M	2.34				0.40				0.007	86.454			
D0140	144hr	4/M	2.71				0.81				0.010	72.214			
D0141	144hr	4/M	2.38				0.45				0.007	71.321			
D0142	144hr	4/M	3.51	2.73	0.541	0.271	0.43	0.52	0.192	0.096	0.010	64.952	73.735	9.075	4.537
D0135	168hr	3/F	2.08				0.68				0.007	68.247			
D0136	168hr	3/F	2.23				1.01				0.009	80.640			
D0137	168hr	3/F	2.45				0.69				0.011	82.314			
D0138	168hr	3/F	4.05	2.70	0.913	0.456	2.49	1.22	0.862	0.431	0.014	56.907	72.027	11.873	5.937
D0139	168hr	4/M	1.80				0.40				0.006	86.460			
D0140	168hr	4/M	2.18				0.47				0.009	72.223			
D0141	168hr	4/M	1.79				0.45				0.006	71.327			
D0142	168hr	4/M	3.27	2.26	0.696	0.348	0.49	0.45	0.039	0.020	0.008	64.960	73.742	9.074	4.537

D0135 24hr. Concentration for radioactivity and parent µg D6/g of feces excluded, due to possible incorrect feces weight.

Expired Volatiles

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 µCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

Charcoal

Animal ID	Time Point	Group ID/ Sex	Radioactivity Conc. µg Eq D_6/hr of Expired Volatiles	Avg. Conc. µg Eq D_6/hr of Expired Volatiles	SD	SEM	Parent µg D_6/hr Expired Volatiles	Parent µg D_6/hr Average/ sex/ timepoint	SD	SEM	% Dose Recovered in Expired Volatiles	Cum. % Dose Recovered in Expired Volatiles	Avg. Cum. % Dose Recovered per Group	SD	SEM
D0135	1	3/F	51.50				43.24				0.034	0.034			
D0136	1	3/F	28.79				24.01				0.019	0.019			
D0137	1	3/F	255.49				216.00				0.172	0.172			
D0138	1	3/F	57.05	98.21	105.566	52.783	47.03	82.57	89.523	44.762	0.039	0.039	0.066	0.071	0.036
D0139	1	4/M	165.83				139.34				0.082	0.082			
D0140	1	4/M	25.09				21.23				0.014	0.014			
D0141	1	4/M	15.02				11.84				0.008	0.008			
D0142	1	4/M	290.12	124.01	130.393	65.196	248.48	105.22	111.748	55.874	0.139	0.139	0.061	0.062	0.031
D0135	2	3/F	15.63				13.81				0.010	0.044			
D0136	2	3/F	5.49				4.94				0.004	0.023			
D0137	2	3/F	122.64				104.82				0.083	0.255			
D0138	2	3/F	20.88	41.16	54.695	27.348	17.78	35.34	46.631	23.315	0.014	0.053	0.094	0.108	0.054
D0139	2	4/M	31.93				27.64				0.016	0.098			
D0140	2	4/M	9.05				9.92				0.005	0.019			
D0141	2	4/M	3.26				3.40				0.002	0.009			
D0142	2	4/M	200.31	61.14	93.605	46.802	173.15	53.53	80.404	40.202	0.096	0.235	0.090	0.104	0.052
D0135	4	3/F	4.69				4.73				0.006	0.050			
D0136	4	3/F	1.24				1.51				0.002	0.025			
D0137	4	3/F	34.08				48.20				0.046	0.300			
D0138	4	3/F	6.53	11.64	15.124	7.562	6.49	15.23	22.075	11.037	0.009	0.062	0.109	0.128	0.064
D0139	4	4/M	11.83				9.77				0.012	0.110			
D0140	4	4/M	3.59				3.35				0.004	0.023			
D0141	4	4/M	1.47				3.08				0.002	0.011			
D0142	4	4/M	68.90	21.45	31.947	15.973	73.92	22.53	34.401	17.200	0.066	0.300	0.111	0.134	0.067
D0135	6	3/F	129.20				126.53				0.169	0.219			
D0136	6	3/F	0.66				0.56				0.001	0.025			
D0137	6	3/F	13.07				12.39				0.018	0.318			
D0138	6	3/F	2.57	36.38	62.123	31.062	2.48	35.49	60.915	30.457	0.003	0.065	0.157	0.136	0.068
D0139	6	4/M	4.41				3.76				0.004	0.114			
D0140	6	4/M	2.41				1.39				0.003	0.026			
D0141	6	4/M	0.60				0.55				0.001	0.012			
D0142	6	4/M	23.72	7.79	10.735	5.368	20.81	6.63	9.551	4.776	0.023	0.323	0.119	0.144	0.072
D0135	12	3/F	148.51				233.39				0.582	0.801			
D0136	12	3/F	15.57				14.62				0.062	0.088			
D0137	12	3/F	80.37				117.79				0.325	0.643			
D0138	12	3/F	1275.92	380.09	599.679	299.840	1180.82	386.66	536.930	268.465	5.200	5.266	1.699	2.397	1.199

Rounding differences may occur due to electronic handling of numbers

Expired Volatiles

Charcoal

Animal ID	Time Point	Group ID/ Sex	Radioactivity Conc. µg Eq D_6/hr of Expired Volatiles	Avg. Conc. µg Eq D_6/hr of Expired Volatiles	SD	SEM	Parent µg D_6/hr Expired Volatiles	Parent µg D_6/hr Average/ sex/ timepoint	SD	SEM	% Dose Recovered in Expired Volatiles	Cum. % Dose Recovered in Expired Volatiles	Avg. Cum. % Dose Recovered per Group	SD	SEM
D0139	12	4/M	153.49				217.45				0.456	0.570			
D0140	12	4/M	321.28				518.87				1.086	1.112			
D0141	12	4/M	98.34				89.96				0.303	0.314			
D0142	12	4/M	392.89	241.50	138.472	69.236	365.88	298.04	185.434	92.717	1.127	1.450	0.862	0.515	0.257
D0135	24	3/F	675.42				608.20				5.294	6.095			
D0136	24	3/F	682.54				616.94				5.457	5.545			
D0137	24	3/F	356.17				297.27				2.878	3.521			
D0138	24	3/F	1409.58	780.93	445.882	222.941	1261.65	696.02	405.341	202.671	11.490	16.756	7.979	5.955	2.977
D0139	24	4/M	502.76				452.88				2.987	3.557			
D0140	24	4/M	1420.17				1257.42				9.602	10.714			
D0141	24	4/M	1031.30				911.90				6.346	6.660			
D0142	24	4/M	1043.11	999.34	377.107	188.553	933.76	888.99	330.886	165.443	5.986	7.436	7.092	2.940	1.470
D0135	48	3/F	75.83				81.73				1.189	7.283			
D0136	48	3/F	116.53				128.13				1.863	7.408			
D0137	48	3/F	18.98				25.75				0.307	3.827			
D0138	48	3/F	300.49	127.96	121.782	60.891	311.57	136.79	123.806	61.903	4.899	21.655	10.043	7.917	3.958
D0139	48	4/M	264.18				259.09				3.139	6.695			
D0140	48	4/M	303.58				288.84				4.105	14.819			
D0141	48	4/M	440.71				411.07				5.423	12.083			
D0142	48	4/M	202.17	302.66	101.058	50.529	327.63	321.66	65.882	32.941	2.320	9.756	10.838	3.451	1.726
D0135	72	3/F	15.93				14.28				0.250	7.533			
D0136	72	3/F	15.57				12.79				0.249	7.657			
D0137	72	3/F	0.89				0.80				0.014	3.842			
D0138	72	3/F	105.82	34.55	48.024	24.012	96.06	30.98	43.800	21.900	1.725	23.380	10.603	8.700	4.350
D0139	72	4/M	6.15				5.34				0.073	6.768			
D0140	72	4/M	29.61				26.24				0.400	15.220			
D0141	72	4/M	41.49				36.98				0.511	12.594			
D0142	72	4/M	11.57	22.21	16.306	8.153	11.05	19.90	14.403	7.201	0.133	9.889	11.118	3.625	1.813
D0135	96	3/F	3.74				3.19				0.059	7.592			
D0136	96	3/F	8.37				7.36				0.134	7.791			
D0137	96	3/F	0.53				0.44				0.009	3.850			
D0138	96	3/F	54.73	16.84	25.461	12.730	50.32	15.33	23.501	11.751	0.892	24.272	10.876	9.113	4.556
D0139	96	4/M	1.80				1.56				0.021	6.790			
D0140	96	4/M	3.75				3.22				0.051	15.270			
D0141	96	4/M	3.86				3.35				0.047	12.641			
D0142	96	4/M	1.91	2.83	1.129	0.564	1.75	2.47	0.946	0.473	0.022	9.911	11.153	3.640	1.820
D0135	120	3/F	1.44				1.33				0.023	7.614			
D0136	120	3/F	6.07				5.56				0.097	7.888			
D0137	120	3/F	0.29				0.26				0.005	3.855			

Rounding differences may occur due to electronic handling of numbers

Expired Volatiles

Charcoal

Animal ID	Time Point	Group ID/ Sex	Radioactivity Conc. µg Eq D_6/hr of Expired Volatiles	Avg. Conc. µg Eq D_6/hr of Expired Volatiles	SD	SEM	Parent µg D_6/hr Expired Volatiles	Parent µg D_6/hr Average/ sex/ timepoint	SD	SEM	% Dose Recovered in Expired Volatiles	Cum. % Dose Recovered in Expired Volatiles	Avg. Cum. % Dose Recovered per Group	SD	SEM
D0138	120	3/F	31.03	9.71	14.434	7.217	27.27	8.60	12.654	6.327	0.506	24.778	11.034	9.346	4.673
D0139	120	4/M	0.92				0.81				0.011	6.801			
D0140	120	4/M	2.61				2.22				0.035	15.306			
D0141	120	4/M	2.19				1.91				0.027	12.668			
D0142	120	4/M	1.12	1.71	0.822	0.411	0.99	1.48	0.687	0.343	0.013	9.924	11.174	3.651	1.826
D0135	144	3/F	0.89				0.80				0.014	7.628			
D0136	144	3/F	5.98				5.23				0.096	7.984			
D0137	144	3/F	0.30				0.26				0.005	3.860			
D0138	144	3/F	18.50	6.42	8.451	4.226	17.25	5.89	7.898	3.949	0.302	25.079	11.138	9.480	4.740
D0139	144	4/M	0.60				0.56				0.007	6.808			
D0140	144	4/M	1.53				1.36				0.021	15.326			
D0141	144	4/M	1.49				1.41				0.018	12.686			
D0142	144	4/M	0.80	1.10	0.474	0.237	0.69	1.00	0.442	0.221	0.009	9.933	11.188	3.658	1.829
D0135	168	3/F	0.41				0.34				0.006	7.635			
D0136	168	3/F	5.06				4.46				0.081	8.064			
D0137	168	3/F	0.01				0.01				0.000	3.860			
D0138	168	3/F	12.56	4.51	5.835	2.917	11.17	3.99	5.192	2.596	0.205	25.284	11.211	9.571	4.785
D0139	168	4/M	0.44				0.42				0.005	6.813			
D0140	168	4/M	0.89				0.83				0.012	15.338			
D0141	168	4/M	1.13				0.99				0.014	12.700			
D0142	168	4/M	0.66	0.78	0.297	0.148	0.59	0.71	0.254	0.127	0.008	9.940	11.198	3.661	1.831

Rounding differences may occur due to electronic handling of numbers

Urine

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 µCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

Urine

Animal ID	Time Point	Group ID/ Sex	Cumulative µg Eq D_6/g of Urine	Avg. Conc. µg Eq D_6/g of Urine	SD	SEM	% Dose Recovered in Urine	Cum. % Dose Recovered in Urine	Avg. Cum. % Dose Recovered per Group	SD	SEM
D0135	6hr	3/F	2.61				0.002	0.002			
D0136	6hr	3/F	0.45				0.000	0.000			
D0137	6hr	3/F	0.24				0.000	0.000			
D0138	6hr	3/F	7.22	2.63	3.245	1.622	0.005	0.005	0.002	0.002	0.001
D0139	6hr	4/M	0.00				0.000	0.000			
D0140	6hr	4/M	5.15				0.003	0.003			
D0141	6hr	4/M	0.00				0.000	0.000			
D0142	6hr	4/M	0.72	1.47	2.480	1.240	0.000	0.000	0.001	0.001	0.001
D0135	12hr	3/F	27.73				0.018	0.020			
D0136	12hr	3/F	42.31				0.028	0.028			
D0137	12hr	3/F	33.03				0.022	0.022			
D0138	12hr	3/F	31.19	33.56	6.233	3.116	0.021	0.026	0.024	0.004	0.002
D0139	12hr	4/M	75.08				0.037	0.037			
D0140	12hr	4/M	32.95				0.019	0.021			
D0141	12hr	4/M	69.08				0.035	0.035			
D0142	12hr	4/M	94.89	68.00	25.840	12.920	0.045	0.046	0.035	0.010	0.005
D0135	24hr	3/F	100.55				0.066	0.085			
D0136	24hr	3/F	108.63				0.072	0.101			
D0137	24hr	3/F	104.14				0.070	0.093			
D0138	24hr	3/F	85.27	99.65	10.136	5.068	0.058	0.084	0.091	0.008	0.004
D0139	24hr	4/M	252.25				0.125	0.162			
D0140	24hr	4/M	202.66				0.114	0.136			
D0141	24hr	4/M	218.22				0.112	0.147			
D0142	24hr	4/M	188.28	215.35	27.468	13.734	0.090	0.136	0.145	0.012	0.006
D0135	48hr	3/F	218.62				0.143	0.228			
D0136	48hr	3/F	146.65				0.098	0.199			
D0137	48hr	3/F	279.73				0.188	0.281			
D0138	48hr	3/F	239.61	221.15	55.767	27.883	0.163	0.247	0.239	0.034	0.017
D0139	48hr	4/M	227.06				0.112	0.274			
D0140	48hr	4/M	310.09				0.175	0.310			
D0141	48hr	4/M	272.39				0.140	0.287			
D0142	48hr	4/M	397.59	301.78	72.329	36.164	0.190	0.326	0.299	0.023	0.012
D0135	72hr	3/F	63.35				0.041	0.270			
D0136	72hr	3/F	48.60				0.032	0.231			

Urine

Urine

Animal ID	Time Point	Group ID/ Sex	Cumulative µg Eq D_6/g of Urine	Avg. Conc. µg Eq D_6/g of Urine	SD	SEM	% Dose Recovered in Urine	Cum. % Dose Recovered in Urine	Avg. Cum. % Dose Recovered per Group	SD	SEM
D0137	72hr	3/F	77.35				0.052	0.333			
D0138	72hr	3/F	65.18	63.62	11.786	5.893	0.044	0.291	0.281	0.043	0.021
D0139	72hr	4/M	57.86				0.029	0.303			
D0140	72hr	4/M	70.67				0.040	0.350			
D0141	72hr	4/M	82.17				0.042	0.329			
D0142	72hr	4/M	99.39	77.52	17.638	8.819	0.048	0.373	0.339	0.030	0.015
D0135	96hr	3/F	28.71				0.019	0.288			
D0136	96hr	3/F	21.25				0.014	0.245			
D0137	96hr	3/F	24.18				0.016	0.349			
D0138	96hr	3/F	30.58	26.18	4.245	2.122	0.021	0.312	0.299	0.044	0.022
D0139	96hr	4/M	25.59				0.013	0.316			
D0140	96hr	4/M	30.22				0.017	0.367			
D0141	96hr	4/M	29.97				0.015	0.344			
D0142	96hr	4/M	34.44	30.05	3.618	1.809	0.016	0.390	0.354	0.032	0.016
D0135	120hr	3/F	17.04				0.011	0.300			
D0136	120hr	3/F	11.55				0.008	0.253			
D0137	120hr	3/F	15.32				0.010	0.360			
D0138	120hr	3/F	13.86	14.44	2.324	1.162	0.009	0.321	0.308	0.045	0.022
D0139	120hr	4/M	17.52				0.009	0.324			
D0140	120hr	4/M	18.86				0.011	0.378			
D0141	120hr	4/M	17.46				0.009	0.353			
D0142	120hr	4/M	30.71	21.14	6.418	3.209	0.015	0.405	0.365	0.034	0.017
D0135	144hr	3/F	12.14				0.008	0.307			
D0136	144hr	3/F	9.08				0.006	0.259			
D0137	144hr	3/F	11.65				0.008	0.367			
D0138	144hr	3/F	13.38	11.56	1.810	0.905	0.009	0.330	0.316	0.045	0.023
D0139	144hr	4/M	13.56				0.007	0.331			
D0140	144hr	4/M	13.66				0.008	0.386			
D0141	144hr	4/M	12.06				0.006	0.360			
D0142	144hr	4/M	18.91	14.55	2.998	1.499	0.009	0.414	0.372	0.035	0.018
D0135	168hr	3/F	9.81				0.006	0.314			
D0136	168hr	3/F	6.24				0.004	0.263			
D0137	168hr	3/F	9.36				0.006	0.374			
D0138	168hr	3/F	10.80	9.05	1.968	0.984	0.007	0.338	0.322	0.046	0.023
D0139	168hr	4/M	11.62				0.006	0.337			
D0140	168hr	4/M	12.00				0.007	0.392			
D0141	168hr	4/M	10.42				0.005	0.365			
D0142	168hr	4/M	8.26	10.58	1.683	0.841	0.004	0.418	0.378	0.035	0.017

Cage Rinse and GI Content

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 µCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

Cage Rinses

Animal ID	Time Point	Group ID /Sex	Conc. µg Eq D_6/g Cage Rinse	Avg. Conc. µg Eq D_6/g Cage Rinse	SD	SEM	% Dose Recovered in Cage Rinse	Avg. % Dose Recovered per Group	SD	SEM
D0135	168hr	3/F	0.249				0.013			
D0136	168hr	3/F	0.633				0.031			
D0137	168hr	3/F	0.106				0.005			
D0138	168hr	3/F	1.513	0.626	0.632	0.316	0.080	0.032	0.034	0.017
D0139	168hr	4/M	0.266				0.010			
D0140	168hr	4/M	0.458				0.019			
D0141	168hr	4/M	0.236				0.010			
D0142	168hr	4/M	0.355	0.329	0.100	0.050	0.015	0.013	0.004	0.002

GI Content

Animal ID	Time Point	Group ID/ Sex	Conc. µg Eq D_6/g of GI contents	Avg. Conc. µg Eq D_6/g of GI contents	SD	SEM	% Dose Recovered in GI contents	Avg. % Dose Recovered per Group	SD	SEM
D0135	168hr	3/F	0.541				0.003			
D0136	168hr	3/F	0.813				0.005			
D0137	168hr	3/F	0.406				0.003			
D0138	168hr	3/F	1.020	0.695	0.275	0.138	0.006	0.004	0.001	0.001
D0139	168hr	4/M	0.617				0.002			
D0140	168hr	4/M	0.741				0.004			
D0141	168hr	4/M	0.289				0.002			
D0142	168hr	4/M	0.671	0.579	0.200	0.100	0.003	0.003	0.001	0.000

Rounding differences may occur due to electronic handling of numbers

KOH

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 µCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

KOH

Animal ID	Time Point	Group ID/ Sex	Conc. µg Eq D_6/g of KOH	Average Conc. µg Eq D_6/g of KOH	SD	SEM	% Dose Recovered in KOH	Cumulative % Dose Recovered in KOH	Average Cumulative % Dose Recovered per Group	SD	SEM
D0135	24hr	3/F	0.42				0.035	0.035			
D0136	24hr	3/F	0.39				0.036	0.036			
D0137	24hr	3/F	0.47				0.044	0.044			
D0138	24hr	3/F	0.48	0.44	0.043	0.022	0.043	0.043	0.040	0.005	0.002
D0139	24hr	4/M	1.11				0.073	0.073			
D0140	24hr	4/M	0.63				0.055	0.055			
D0141	24hr	4/M	0.94				0.066	0.066			
D0142	24hr	4/M	0.96	0.91	0.200	0.100	0.061	0.061	0.064	0.008	0.004
D0135	48hr	3/F	0.44				0.036	0.071			
D0136	48hr	3/F	0.24				0.021	0.057			
D0137	48hr	3/F	0.59				0.052	0.096			
D0138	48hr	3/F	0.40	0.41	0.142	0.071	0.036	0.080	0.076	0.016	0.008
D0139	48hr	4/M	0.50				0.031	0.104			
D0140	48hr	4/M	0.55				0.045	0.100			
D0141	48hr	4/M	0.53				0.039	0.104			
D0142	48hr	4/M	0.65	0.56	0.064	0.032	0.037	0.098	0.102	0.003	0.002
D0135	72hr	3/F	0.12				0.010	0.081			
D0136	72hr	3/F	0.09				0.008	0.065			
D0137	72hr	3/F	0.16				0.015	0.110			
D0138	72hr	3/F	0.10	0.12	0.029	0.015	0.009	0.089	0.086	0.019	0.010
D0139	72hr	4/M	0.16				0.011	0.115			
D0140	72hr	4/M	0.13				0.010	0.110			
D0141	72hr	4/M	0.20				0.014	0.119			
D0142	72hr	4/M	0.18	0.17	0.029	0.014	0.010	0.109	0.113	0.005	0.002
D0135	96hr	3/F	0.05				0.005	0.086			
D0136	96hr	3/F	0.02				0.002	0.066			
D0137	96hr	3/F	0.04				0.004	0.114			

Rounding differences may occur due to electronic handling of numbers

KOH

KOH

Animal ID	Time Point	Group ID/ Sex	Conc. µg Eq D_6/g of KOH	Average Conc. µg Eq D_6/g of KOH	SD	SEM	% Dose Recovered in KOH	Cumulative % Dose Recovered in KOH	Average Cumulative % Dose Recovered per Group	SD	SEM
D0138	96hr	3/F	0.04	0.04	0.015	0.007	0.004	0.093	0.090	0.020	0.010
D0139	96hr	4/M	0.07				0.004	0.120			
D0140	96hr	4/M	0.06				0.005	0.115			
D0141	96hr	4/M	0.07				0.005	0.124			
D0142	96hr	4/M	0.10	0.07	0.017	0.008	0.007	0.115	0.118	0.004	0.002
D0135	120hr	3/F	0.03				0.003	0.089			
D0136	120hr	3/F	0.02				0.002	0.068			
D0137	120hr	3/F	0.02				0.002	0.116			
D0138	120hr	3/F	0.01	0.02	0.008	0.004	0.001	0.094	0.092	0.020	0.010
D0139	120hr	4/M	0.04				0.003	0.123			
D0140	120hr	4/M	0.04				0.003	0.118			
D0141	120hr	4/M	0.04				0.003	0.127			
D0142	120hr	4/M	0.06	0.04	0.008	0.004	0.003	0.118	0.121	0.004	0.002
D0135	144hr	3/F	0.00				0.000	0.089			
D0136	144hr	3/F	0.00				0.000	0.068			
D0137	144hr	3/F	0.02				0.002	0.118			
D0138	144hr	3/F	0.01	0.01	0.008	0.004	0.001	0.095	0.092	0.021	0.010
D0139	144hr	4/M	0.01				0.001	0.124			
D0140	144hr	4/M	0.01				0.001	0.119			
D0141	144hr	4/M	0.03				0.002	0.129			
D0142	144hr	4/M	0.05	0.03	0.016	0.008	0.003	0.121	0.123	0.004	0.002
D0135	168hr	3/F	0.00				0.000	0.089			
D0136	168hr	3/F	0.00				0.000	0.068			
D0137	168hr	3/F	0.01				0.001	0.119			
D0138	168hr	3/F	0.01	0.01	0.004	0.002	0.001	0.096	0.093	0.021	0.010
D0139	168hr	4/M	0.02				0.002	0.125			
D0140	168hr	4/M	0.02				0.001	0.120			
D0141	168hr	4/M	0.02				0.001	0.130			
D0142	168hr	4/M	0.03	0.02	0.007	0.003	0.002	0.123	0.125	0.004	0.002

Rounding differences may occur due to electronic handling of numbers

Organs and Tissues

Dosing Solution : ^{14}C-D_6 in corn oil

Dosing Solution Specific Activity= 0.032 mCi/mg and 71040 DPM/mg

D_6 Concentration = 119.89 mg D_6/g dosing solution and 592543 DPM/mg D_6 in dosing solution

	Group 3, Females				Group 4, Males			
Tissues	**D0135**	**D0136**	**D0137**	**D0138**	**D0139**	**D0140**	**D0141**	**D0142**
LUNGS								
Concentration µg Eq D_6/g lungs	0.057	1.478	1.827	2.100	1.500	1.682	1.747	1.755
Average/Sex	1.365				1.671			
SD	0.909				0.119			
SEM	0.454				0.059			
% Dose Recovered in lungs	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Average/Sex	0.001				0.001			
SD	0.000				0.000			
SEM	0.000				0.000			
SPLEEN								
Concentration µg Eq D_6/g spleen	1.169	1.244	1.827	1.601	1.468	1.539	1.337	2.298
Average/Sex	1.460				1.661			
SD	0.309				0.433			
SEM	0.154				0.216			
% Dose Recovered in spleen	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.001
Average/Sex	0.000				0.000			
SD	0.000				0.000			
SEM	0.000				0.000			

Organs and Tissues

Tissues	Group 3, Females				Group 4, Males			
	D0135	**D0136**	**D0137**	**D0138**	**D0139**	**D0140**	**D0141**	**D0142**
LIVER								
Concentration µg Eq D_6/g liver	8.182	6.200	9.180	7.891	4.589	4.475	5.429	7.497
Average/Sex	7.863				5.498			
SD	1.239				1.399			
SEM	0.619				0.700			
% Dose Recovered in liver	0.030	0.023	0.038	0.032	0.019	0.021	0.022	0.027
Average/Sex	0.031				0.022			
SD	0.006				0.004			
SEM	0.003				0.002			
PERIRENAL FAT								
Concentration µg Eq D_6/g fat	4.99	4.74	5.01	5.75	3.31	3.45	3.06	5.93
Average/Sex	5.122				3.939			
SD	0.434				1.338			
SEM	0.217				0.669			
% Dose Recovered in fat	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Average/Sex	0.001				0.001			
SD	0.000				0.000			
SEM	0.000				0.000			
ADRENALS								
Concentration µg Eq D_6/g adrenals	11.014	9.470	11.183	12.889	4.106	4.671	5.219	4.451
Average/Sex	11.139				4.612			
SD	1.398				0.467			
SEM	0.699				0.233			
% Dose Recovered in adrenals	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000
Average/Sex	0.001				0.000			
SD	0.000				0.000			
SEM	0.000				0.000			

Rounding differences may occur due to electronic handling of numbers

Organs and Tissues

Tissues	Group 3, Females				Group 4, Males			
	D0135	**D0136**	**D0137**	**D0138**	**D0139**	**D0140**	**D0141**	**D0142**
KIDNEYS								
Concentration µg Eq D_6/g kidneys	3.712	2.604	3.325	3.540	1.675	2.688	1.911	3.669
Average/Sex	3.295				2.486			
SD	0.488				0.900			
SEM	0.244				0.450			
% Dose Recovered in kidneys	0.003	0.002	0.003	0.003	0.001	0.003	0.002	0.003
Average/Sex	0.003				0.002			
SD	0.000				0.001			
SEM	0.000				0.000			
TESTES or OVARIES	**Ovaries**				**Testes**			
Concentration µg Eq D_6/g ovaries or testes	10.579	6.436	7.682	8.208	0.622	0.707	0.674	0.927
Average/Sex	8.226				0.732			
SD	1.736				0.134			
SEM	0.868				0.067			
% Dose Recovered in ovaries or testes	0.001	0.000	0.000	0.000	0.001	0.001	0.001	0.001
Average/Sex	0.000				0.001			
SD	0.000				0.000			
SEM	0.000				0.000			
EMPTIED GI TRACT								
Concentration µg Eq D_6/g GI tract	1.499	1.321	1.516	1.728	1.217	1.390	1.139	1.817
Average/Sex	1.516				1.391			
SD	0.166				0.303			
SEM	0.083				0.152			
% Dose Recovered in GI tract	0.006	0.005	0.007	0.007	0.005	0.005	0.004	0.006
Average/Sex	0.007				0.005			
SD	0.001				0.001			
SEM	0.000				0.000			

Rounding differences may occur due to electronic handling of numbers

Organs and Tissues

Tissues	Group 3, Females				Group 4, Males			
	D0135	**D0136**	**D0137**	**D0138**	**D0139**	**D0140**	**D0141**	**D0142**
REMAINING CARCASS								
Concentration µg Eq D_6/g carcass	1.917	1.579	2.141	2.179	1.566	1.689	1.578	1.937
Average/Sex	1.954				1.692			
SD	0.275				0.172			
SEM	0.138				0.086			
% Dose Recovered in carcass	0.161	0.134	0.184	0.180	0.132	0.159	0.137	0.149
Average/Sex	0.165				0.144			
SD	0.023				0.012			
SEM	0.011				0.006			

Cumulative Tables

Percent of administered dose recovered in urine, expired volatiles, CO2 ,tissues and remaining carcasses (% Dose absorbed)

Fischer 344, Females

Animal ID	Group	% of Administered dose: Urine	Expired volatiles	CO_2 (KOH)	Carcass	Adrenal	Lung	Ovaries	Fat	Spleen	Kidneys	Liver	GI tract	Tissues	% Dose absorbed
D0135	3	0.314	7.635	0.089	0.161	0.001	0.000	0.001	0.001	0.000	0.003	0.030	0.006	0.042	8.241
D0136	3	0.263	8.064	0.068	0.134	0.001	0.001	0.000	0.001	0.000	0.002	0.023	0.005	0.033	8.563
D0137	3	0.374	3.860	0.119	0.184	0.001	0.001	0.000	0.001	0.001	0.003	0.038	0.007	0.051	4.587
D0138	3	0.338	25.284	0.096	0.180	0.001	0.001	0.000	0.001	0.000	0.003	0.032	0.007	0.046	25.943
	Avg.	**0.322**	**11.211**	**0.093**	**0.165**	**0.001**	**0.001**	**0.000**	**0.001**	**0.000**	**0.003**	**0.031**	**0.007**	**0.043**	**11.834**
	SD	**0.046**	**9.571**	**0.021**	**0.023**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.006**	**0.001**	**0.008**	**9.578**
	SEM	**0.023**	**4.785**	**0.010**	**0.011**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.003**	**0.000**	**0.004**	**4.789**

Fischer 344, Males

Animal ID	Group	% of Administered dose: Urine	Expired volatiles	CO_2 (KOH)	Carcass	Adrenal	Lung	Testes	Fat	Spleen	Kidneys	Liver	GI tract	Tissues	% Dose absorbed
D0139	4	0.337	6.813	0.125	0.132	0.000	0.001	0.001	0.001	0.000	0.001	0.019	0.005	0.027	7.434
D0140	4	0.392	15.338	0.120	0.159	0.000	0.001	0.001	0.001	0.000	0.003	0.021	0.005	0.033	16.043
D0141	4	0.365	12.700	0.130	0.137	0.000	0.001	0.001	0.001	0.000	0.002	0.022	0.004	0.031	13.363
D0142	4	0.418	9.940	0.123	0.149	0.000	0.001	0.001	0.001	0.001	0.003	0.027	0.006	0.040	10.670
	Avg.	**0.378**	**11.198**	**0.125**	**0.144**	**0.000**	**0.001**	**0.001**	**0.001**	**0.000**	**0.002**	**0.022**	**0.005**	**0.033**	**11.878**
	SD	**0.035**	**3.661**	**0.004**	**0.012**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.004**	**0.001**	**0.005**	**3.686**
	SEM	**0.017**	**1.831**	**0.002**	**0.006**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**	**0.003**	**1.843**

Percent of administered dose recovered in excreta

Fischer 344, Females

Animal ID	Group	% of Administered dose: Feces[1]	Contents of GI tract	Cage rinse	Eliminated through GI Tract
D0135	3	78.445	0.003	0.013	78.461
D0136	3	92.690	0.005	0.031	92.725
D0137	3	94.614	0.003	0.005	94.622
D0138	3	65.410	0.006	0.080	65.496
	Avg.	**82.790**	**0.004**	**0.032**	**82.826**
	SD	**13.647**	**0.001**	**0.034**	**13.620**
	SEM	**6.824**	**0.001**	**0.017**	**6.810**

Fischer 344, Males

Animal ID	Group	% of Administered dose: Feces[1)]	Contents of GI tract	Cage rinse	Eliminated through GI Tract
D0139	4	99.379	0.002	0.010	99.392
D0140	4	83.014	0.004	0.019	83.037
D0141	4	81.985	0.002	0.010	81.996
D0142	4	74.666	0.003	0.015	74.684
	Avg.	**84.761**	**0.003**	**0.013**	**84.777**
	SD	**10.430**	**0.001**	**0.004**	**10.428**
	SEM	**5.215**	**0.000**	**0.002**	**5.214**

[1)] Percent dose recovered in feces was corrected for the sample processing efficiency (87%)

Mass balance as a percent of administered dose

Fischer 344, Females

		% of Administered dose						
Animal ID	Group	% Dose eliminated through GI Tract[1)]	% Dose in urine	% Dose in expired volatiles	% Dose in KOH	%Dose in tissues and carcass	% Dose absorbed	Total Recovery[1] (%)
D0135	3	78.461	0.314	7.635	0.089	0.203	8.241	86.702
D0136	3	92.725	0.263	8.064	0.068	0.167	8.563	101.288
D0137	3	94.622	0.374	3.860	0.119	0.235	4.587	99.209
D0138	3	65.496	0.338	25.284	0.096	0.226	25.943	91.439
	Avg.	**82.826**	**0.322**	**11.211**	**0.093**	**0.208**	**11.834**	**94.660**
	SD	**13.620**	**0.046**	**9.571**	**0.021**	**0.030**	**9.578**	**6.791**
	SEM	**6.810**	**0.023**	**4.785**	**0.010**	**0.015**	**4.789**	**3.395**

[1)] Percent dose recovered in feces was corrected for the sample processing efficiency (87%)

Fischer 344, Males

		% of Administered dose						
Animal ID	Group	% Dose eliminated through GI Tract[1)]	% Dose in urine	% Dose in expired volatiles	% Dose in KOH	%Dose in tissues and carcass	% Dose absorbed	Total Recovery[1] (%)
D0139	4	99.392	0.337	6.813	0.125	0.160	7.434	106.826
D0140	4	83.037	0.392	15.338	0.120	0.193	16.043	99.080
D0141	4	81.996	0.365	12.700	0.130	0.168	13.363	95.359
D0142	4	74.684	0.418	9.940	0.123	0.189	10.670	85.354
	Avg.	**84.777**	**0.378**	**11.198**	**0.125**	**0.177**	**11.878**	**96.655**
	SD	**10.428**	**0.035**	**3.661**	**0.004**	**0.016**	**3.686**	**8.921**
	SEM	**5.214**	**0.017**	**1.831**	**0.002**	**0.008**	**1.843**	**4.460**

[1)] Percent dose recovered in feces was corrected for the sample processing efficiency (87%)

Mass balance as a percent of the total recovered dose

Fischer 344, Females

Animal ID	Group	Total Recovery[1] (% of admin. dose)	% of Total Recovered Dose: Eliminated through GI Tract [1)]	Urine	Expired volatiles	KOH	Tissues, carcass	% of Total Recovery Absorbed
D0135	3	86.702	90.495	0.362	8.806	0.103	0.234	9.505
D0136	3	101.288	91.546	0.260	7.962	0.067	0.165	8.454
D0137	3	99.209	95.376	0.377	3.890	0.120	0.237	4.624
D0138	3	91.439	71.628	0.369	27.651	0.104	0.247	28.372
Avg.		**94.660**	**87.261**	**0.342**	**12.077**	**0.099**	**0.221**	**12.739**
SD		**6.791**	**10.631**	**0.055**	**10.602**	**0.022**	**0.037**	**10.631**
SEM		**3.395**	**5.316**	**0.028**	**5.301**	**0.011**	**0.019**	**5.316**

[1)] Percent dose recovered in feces was corrected for the sample processing efficiency (87%)

Fischer 344, Males

Animal ID	Group	Total Recovery[1] (% of admin. dose)	% of Total Recovered Dose: Eliminated through GI Tract [1)]	Urine	Expired volatiles	KOH	Tissues, carcass	% of Total Recovery Absorbed
D0139	4	106.826	93.041	0.315	6.378	0.117	0.149	6.959
D0140	4	99.080	83.808	0.396	15.481	0.121	0.194	16.192
D0141	4	95.359	85.987	0.383	13.318	0.136	0.176	14.013
D0142	4	85.354	87.499	0.489	11.646	0.144	0.222	12.501
Avg.		**96.655**	**87.584**	**0.396**	**11.706**	**0.130**	**0.185**	**12.416**
SD		**8.921**	**3.941**	**0.072**	**3.883**	**0.013**	**0.030**	**3.941**
SEM		**4.460**	**1.970**	**0.036**	**1.942**	**0.006**	**0.015**	**1.970**

[1)] Percent dose recovered in feces was corrected for the sample processing efficiency (87%)

FECAL PROCESSING EFFICIENCY

Feces Spiking Experiment for determining Processing Efficiency

Feces QC's D_6 Study 9683	dpm/g homogenate	Average dpm/g homogenate	% Relative Range	µCi/g feces	µCi/g feces spiked	% Processing Efficiency	Average % Processing Efficiency
Blank 09/25/02	187						
	123						
CO-1-09/25/02	2708344	2647105	4.63%	5.02	5.69	88%	**87%**
	2585866						
(A) CO-2-09/25/02	*2227720*	*2660933*	*32.56%*	*5.07*	*5.20*	*97%*	
	3094147						
(A) CO-3-09/25/02	*2703264*	*3221145*	*32.16%*	*6.09*	*5.86*	*104%*	
	3739027						
*CO-2-10/1/02	2297193	2266685	2.69%	4.32	5.20	83%	
	2236177						
*CO-3-10/1/02	2778072	2751025	1.97%	5.20	5.86	89%	
	2723978						

* Reanalysis of CO-2 09/25/02 and C0-3 09/25/02.

(*A*) Samples were not used in average due to high % Relative Range.

QUALITATIVE METABOLITE PROFILE ANALYSIS IN URINE AND FECES

HPLC urine analysis

Sample ID	Time Point (hours post dosing)	Group / Sex	NET AREA CPM Methyl-silanetriol **Average 3.4min.** Retention time metabolite	Dimethyl-disiloxane-1,3,3,3-tetrol **Average 4.2min.** Retention time metabolite	Dimethyl-silanediol **Average 14.2min.** Retention time metabolite	Total CPMs Detected	Sum of percentage of (2) most abundant peaks
D0135	12	3/F	467	0	398	865	
			54%		*46%*		100%
D0136	12	3/F	0	0	377	377	
			0%		*100%*		100%
D0137	12	3/F	354	0		354	
			100%		*0%*		100%
D0138	12	3/F	nd	nd	nd	nd	
							0%
D0139	12	4/M	718	0		718	
			100%		*0%*		100%
D0140	12	4/M	997	0	671	1668	
			60%		*40%*		100%
D0141	12	4/M	943	0	575	1518	
			62%		*38%*		100%
D0142	12	4/M	1022	0	581	1603	
			64%		*36%*		100%
D0131	24	1/F					
D0132	24	1/F					
D0133	24	2/M					
D0134	24	2/M					
D0135	24	3/F	1086	0	975	2061	
			53%		*47%*		100%
D0136	24	3/F	522	0	656	1178	
			44%		*56%*		100%

Rounding differences may occur due to electronic handling of numbers

HPLC urine analysis

Sample ID	Time Point (hours post dosing)	Group / Sex	NET AREA CPM Methyl-silanetriol **Average 3.4min.** Retention time metabolite	Dimethyl-disiloxane-1,3,3,3-tetrol **Average 4.2min.** Retention time metabolite	Dimethyl-silanediol **Average 14.2min.** Retention time metabolite	Total CPMs Detected	Sum of percentage of (2) most abundant peaks
D0137	24	3/F	713	0	608	1321	
			54%		*46%*		100%
D0138	24	3/F	729	317	690	1736	
			42%	*18%*	*40%*		82%
D0139	24	4/M	1931	517	1184	3632	
			53%	*14%*	*33%*		86%
D0140	24	4/M	2074	387	1453	3914	
			53%	*10%*	*37%*		90%
D0141	24	4/M	1600	0	1021	2621	
			61%		*39%*		100%
D0142	24	4/M	2388	492	1712	4592	
			52%	*11%*	*37%*		89%
D0135	48	3/F	720	0	827	1547	
			47%		*53%*		100%
D0136	48	3/F	368	0	326	694	
			53%		*47%*		100%
D0137	48	3/F	1058	0	1014	2072	
			51%		*49%*		100%
D0138	48	3/F	1201	436	761	2398	
			50%	*18%*	*32%*		82%
D0139	48	4/M	1012	0	789	1801	
			56%		*44%*		100%
D0140	48	4/M	1591	0	1046	2637	
			95%		*40%*		135%
D0141	48	4/M	2082	0	1209	3291	
			137%		*37%*		174%
D0142	48	4/M	3583	568	2203	6354	
			224%	*35%*	*35%*		258%

Rounding differences may occur due to electronic handling of numbers

QUALITATIVE METABOLITE PROFILE ANALYSIS IN URINE AND FECES

HPLC feces analysis 6 and 12 hr time points

Sample ID	Time Point (hours post dosing)	Group /Sex	Dodecamethyl-cyclohexa-siloxane **Average 55.20 min.** Retention time metabolite	Total CPMs Detected	Sum of percentage of (2) most abundant peaks
14C-D6 Standard	NA	NA	116138 *100%*	116138	100%
D0135	6	3/F	1980 *100%*	1980	100%
14C-D6 Standard	NA	NA	116138 *100%*	116138	100%
D0135	12	3/F	15596 *100%*	15596	100%
D0136	12	3/F	9557 *100%*	9557	100%
D0137	12	3/F	14898 *100%*	14898	100%
D0138	12	3/F	25370 *100%*	25370	100%
D0139	12	4/M	30694 *100%*	30694	100%
D0140	12	4/M	315 *100%*	315	100%
D0141	12	4/M	14066 *100%*	14066	100%
D0142	12	4/M	821 *100%*	821	100%

Rounding differences may occur due to electronic handling of numbers

HPLC feces analysis 24hr time point

Sample ID	Time Point (hours post dosing)	Group /Sex	**Average 1.3** Unknown	**Average 15.30** Unknown	**Average 26.7** Unknown	**Average 43.1** Unknown	**Average 44.1** Unknown	Dodecamethyl-cyclohexa- **Average 45.20 min.** Retention time metabolite	Total CPMs Detected	Sum of percentage of (2) most abundant peaks
14C-D6 Standard	NA	NA				416	435	139132	139983	
						0.30%	*0.31%*	*99.39%*		99.70%
D0131	24	1/F								
D0132	24	1/F								
D0133	24	2/M								
D0134	24	2/M								
D0135	24	3/F				475	1137	115036	116648	
						0.41%	*0.97%*	*98.62%*		99.59%
D0136	24	3/F						86398	86398	
								100.00%		100.00%
D0137	24	3/F					468	52916	53384	
							0.88%	*99.12%*		100.00%
D0138	24	3/F			346		698	53534	54578	
					0.63%		*1.28%*	*98.09%*		99.37%
D0139	24	4/M						46350	46350	
								100.00%		100.00%
D0140	24	4/M		303			936	54589	55828	
				0.56%			*1.68%*	*97.78%*		99.46%
D0141	24	4/M					823	50832	51655	
							1.59%	*98.41%*		100.00%
D0142	24	4/M	639					62070	62709	
			1%					*98.98%*		100.00%

Rounding differences may occur due to electronic handling of numbers

HPLC feces analysis 48hr time point

Sample ID	Time Point (hours post dosing)	Group /Sex	**Average 13.40** Unknown	**Average 44.1** Unknown	Dodecamethyl-cyclohexasiloxane **Average 45.30 min.** Retention time metabolite	Total CPMs Detected	Sum of percentage of (2) most abundant peaks
14C-D6 Standard	NA	NA		334	146960	147294	
				0.23%	*99.77%*		100.00%
D0135	48	3/F		390	34618	35008	
				1.11%	*98.89%*		100.00%
D0136	48	3/F			20959	20959	
					100.00%		100.00%
D0137	48	3/F	363	388	10609	11360	
			3.42%	*3.42%*	*93.39%*		96.80%
D0138	48	3/F			21026	21026	
					100.00%		100.00%
D0139	48	4/M			15029	15029	
					100.00%		100.00%
D0140	48	4/M		660	40292	40952	
				1.61%	*98.39%*		100.00%
D0141	48	4/M		1007	58601	59608	
				1.69%	*98.31%*		100.00%
D0142	48	4/M		640	57686	58326	
				1.10%	*98.90%*		100.00%

Rounding differences may occur due to electronic handling of numbers

HPLC feces analysis 72hr time point

Sample ID	Time Point (hours post dosing)	Group /Sex	Dodecamethyl-cyclohexasiloxane **Average 47.00 min.** Retention time metabolite	Total CPMs Detected	Sum of percentage of (2) most abundant peaks
14C-D6 Standard	NA	NA	118371 *100.00%*	118371	100.00%
D0135	72	3/F	3178 *100.00%*	3178	100.00%
D0136	72	3/F	695 *100.00%*	695	100.00%
D0137	72	3/F	1928 *100.00%*	1928	100.00%
D0138	72	3/F	1559 *100.00%*	1559	100.00%
D0139	72	4/M	360 *100.00%*	360	100.00%
D0140	72	4/M	800 *100.00%*	800	100.00%
D0141	72	4/M	799 *100.00%*	799	100.00%
D0142	72	4/M	4438 *100.00%*	4438	100.00%

PARENT QUALITY CONTROL SAMPLES FOR FECES, EXPIRED VOLATILES AND BLOOD

Blood QC's

Matrix	QC Name	Expected [D_6] ng	[D_6] found ng	%Relative Error (Accuracy)	Average % Relative Error (Accuracy)
Blood	6-19-02 QC 0-A	0.00	N/AP		
Blood	6-19-02 QC 0-B	0.00	N/AP		
Blood	6-19-02 QC 1-A	35	202.97	485%	539%
Blood	6-19-02 QC 1-B	36	246.65	593%	
Blood	6-19-02 QC 2-B	775	799.75	3%	12%
Blood	6-19-02 QC 2-A	775	942.06	22%	
Blood	6-19-02 QC 3-B	4251	4041.59	-5%	-7%
Blood	6-19-02 QC 3-A	4291	3941.90	-8%	
Blood	6-20-02 QC 0-A	0	N/AP		
Blood	6-20-02 QC 0-B	0	N/AP		
Blood	6-20-02 QC 1-A	36	BLQ	N/AP	N/AP
Blood	6-20-02 QC 1-B	36	BLQ	N/AP	
Blood	6-20-02 QC 2-B	834	887.32	6%	4%
Blood	6-20-02 QC 2-A	814	833.86	2%	
Blood	6-20-02 QC 3-B	4271	3980.56	-7%	-7%
Blood	6-20-02 QC 3-A	4271	3966.39	-7%	
Blood	6-21-02 QC 0-A	0	N/AP		
Blood	6-21-02 QC 0-B	0	N/AP		
Blood	6-21-02 QC 1-A	38	117.26	207%	223%
Blood	6-21-02 QC 1-B	36	121.03	240%	
Blood	6-21-02 QC 2-B	814	830.53	2%	7%
Blood	6-21-02 QC 2-A	775	870.78	12%	
Blood	6-21-02 QC 3-B	4330	4098.97	-5%	-6%
Blood	6-21-02 QC 3-A	4330	4035.63	-7%	
Blood	6-22-02 QC 0-A	0	N/AP		
Blood	6-22-02 QC 0-B	0	N/AP		
Blood	6-22-02 QC 1-A	39	108.12	176%	175%
Blood	6-22-02 QC 1-B	39	107.30	174%	
Blood	6-22-02 QC 2-A	834	963.22	15%	13%
Blood	6-22-02 QC 2-B	854	940.38	10%	
Blood	6-22-02 QC 3-A	4251	4253.50	0%	0%
Blood	6-22-02 QC 3-B	4291	4250.41	-1%	

Rounding differences may occur due to electronic handling of numbers

Blood QC's

Matrix	QC Name	Expected [D_6] ng	[D_6] found ng	%Relative Error (Accuracy)	Average % Relative Error (Accuracy)
Blood	6-23-02 QC 0-A	0	N/AP		
Blood	6-23-02 QC 0-B	0	N/AP		
Blood	6-23-02 QC 1-A	36	126.81	248%	233%
Blood	6-23-02 QC 1-B	37	118.90	218%	
Blood	6-23-02 QC 2-A	814	953.88	17%	19%
Blood	6-23-02 QC 2-B	834	1006.63	21%	
Blood	6-23-02 QC 3-A	4251	4307.70	1%	2%
Blood	6-23-02 QC 3-B	4271	4397.94	3%	
Blood	6-24-02 QC 0-A	0	N/AP		
Blood	6-24-02 QC 0-B	0	N/AP		
Blood	6-24-02 QC 1-A	38	111.13	190%	196%
Blood	6-24-02 QC 1-B	36	107.31	202%	
Blood	6-24-02 QC 2-A	814	919.22	13%	17%
Blood	6-24-02 QC 2-B	814	990.60	22%	
Blood	6-24-02 QC 3-A	4271	4569.93	7%	5%
Blood	6-24-02 QC 3-B	4291	4450.14	4%	
Blood	6-25-02 QC 0-A	0	N/AP		
Blood	6-25-02 QC 0-B	0	N/AP		
Blood	6-25-02 QC 1-A	36	113.07	210%	204%
Blood	6-25-02 QC 1-B	38	113.93	198%	
Blood	6-25-02 QC 2-A	834	1015.03	22%	22%
Blood	6-25-02 QC 2-B	834	1014.41	22%	
Blood	6-25-02 QC 3-A	4271	4414.24	3%	4%
Blood	6-25-02 QC 3-B	4291	4482.28	4%	
Blood	6-26-02 QC 0-A	0	N/AP		
Blood	6-26-02 QC 0-B	0	N/AP		
Blood	6-26-02 QC 1-A	36	133.35	275%	293%
Blood	6-26-02 QC 1-B	32	131.77	311%	
Blood	6-26-02 QC 2-A	735	1023.06	39%	36%
Blood	6-26-02 QC 2-B	755	1007.58	33%	
Blood	6-26-02 QC 3-A	4152	4585.16	10%	19%
Blood	6-26-02 QC 3-B	3595	4615.85	28%	

Feces QC's

Event 9683E04b

Matrix	QC Name	Expected [D_6] ng	[D_6] found ng	%Relative Error (Accuracy)	Average % Relative Error (Accuracy)
Feces	7-17-02 QC 0-A	0	N/AP		
Feces	7-17-02 QC 0-B	0	N/AP		
Feces	7-17-02 QC 1-A	45	BLQ	N/AP	N/AP
Feces	7-17-02 QC 1-B	31	BLQ	N/AP	
Feces	7-17-02 QC 2-B	1024	1028.96	0%	1%
Feces	7-17-02 QC 2-A	1052	1070.96	2%	
Feces	7-17-02 QC 3-A	5813	5706.67	-2%	-1%
Feces	7-17-02 QC 3-B	5896	5840.25	-1%	
Feces	7-17-02 QC 4-A	105985	97840.83	-8%	-7%
Feces	7-17-02 QC 4-B	105488	97994.09	-7%	

Event 9683E04c

Feces	7-17-02 QC 0-A	0	N/AP		
Feces	7-17-02 QC 0-B	0	N/AP		
Feces	7-17-02 QC 1-A	45	nd	N/AP	N/AP
Feces	7-17-02 QC 1-B	31	nd	N/AP	
Feces	7-17-02 QC 2-B	1024	1005.53	-2%	4%
Feces	7-17-02 QC 2-A	1052	1145.02	9%	
Feces	7-17-02 QC 3-A	5813	6043.64	4%	3%
Feces	7-17-02 QC 3-B	5896	5980.51	1%	
Feces	7-17-02 QC 4-A	105985	98808.70	-7%	N/AP
Feces	7-17-02 QC 4-B	105488	IE	N/AP	

Event 9683E04d

Feces	7-17-02 QC 0-A	0	N/AP		
Feces	7-17-02 QC 0-B	0	N/AP		
Feces	7-17-02 QC 1-A	45	nd	N/AP	N/AP
Feces	7-17-02 QC 1-B	31	BLQ	N/AP	
Feces	7-17-02 QC 2-B	1024	1058.89	3%	10%
Feces	7-17-02 QC 2-A	1052	1225.96	17%	
Feces	7-17-02 QC 3-A	5813	6048.10	4%	4%
Feces	7-17-02 QC 3-B	5896	6077.91	3%	
Feces	7-17-02 QC 4-A	105985	ALQ	N/AP	N/AP
Feces	7-17-02 QC 4-B	105488	ALQ	N/AP	

Feces QC's

Event 9683E04f

Matrix	QC Name	Expected [D_6] ng	[D_6] found ng	%Relative Error (Accuracy)	Average % Relative Error (Accuracy)
Feces	7-17-02 QC 0-A	0	N/AP		
Feces	7-17-02 QC 0-B	0	N/AP		
Feces	7-17-02 QC 2-B	1024	1010.00	-1%	-1%
Feces	7-17-02 QC 2-A	1052	1051.73	0%	

Event 9683E04h

Feces	7-17-02 QC 0-A	0	N/AP		
Feces	7-17-02 QC 0-B	0	N/AP		
Feces	7-17-02 QC 1-A	45	37.78	-17%	-22%
Feces	7-17-02 QC 1-B	31	22.63	-27%	
Feces	7-17-02 QC 2-B	1024	1000.38	-2%	0%
Feces	7-17-02 QC 2-A	1052	1081.80	3%	

Event 9683E04i

Feces	7-17-02 QC 0-A	0	N/AP		
Feces	7-17-02 QC 0-B	0	N/AP		
Feces	7-17-02 QC 1-A	45	41.60	-8%	-2%
Feces	7-17-02 QC 1-B	31	32.64	5%	

Charcoal QC's (Expired Volatiles)

Event 9683E03a

Matrix	QC Name	Expected [D_6] ng	[D_6] found ng	%Relative Error (Accuracy)	Average % Relative Error (Accuracy)
Charcoal	QC-0ug-a	0			
Charcoal	QC-0ug-b	0			
Charcoal	QC-2ug-a	103	135.00	31%	41%
Charcoal	QC-2ug-b	115	175.49	52%	
Charcoal	QC-50ug-a	2562	2635.23	3%	-6%
Charcoal	QC-50ug-b	2732	2315.16	-15%	
Charcoal	QC-500ug-a	27776	24016.13	-14%	-12%
Charcoal	QC-500ug-b	29168	25893.17	-11%	
Charcoal	QC-2000ug-a	129706	108279.42	-17%	-16%
Charcoal	QC-2000ug-b	129890	108672.32	-16%	

Event 9683E03b

Charcoal	QC-0ug-a	0			
Charcoal	QC-0ug-b	0			
Charcoal	QC-2ug-a	103	95.60	-8%	-8%
Charcoal	QC-2ug-b	115	106.41	-8%	
Charcoal	QC-50ug-a	2562	2281.14	-11%	-10%
Charcoal	QC-50ug-b	2732	2503.13	-8%	
Charcoal	QC-500ug-a	27776	24340.09	-12%	-11%
Charcoal	QC-500ug-b	29168	26465.38	-9%	
Charcoal	QC-2000ug-a	129706	118332.38	-9%	-9%
Charcoal	QC-2000ug-b	129890	119118.92	-8%	

Event 9683E03c

Charcoal	QC-0ug-a	0			
Charcoal	QC-0ug-b	0			
Charcoal	QC-2ug-a	103	89.04	-14%	-14%
Charcoal	QC-2ug-b	115	98.15	-15%	
Charcoal	QC-50ug-a	2562	2393.94	-7%	-8%
Charcoal	QC-50ug-b	2732	2458.80	-10%	
Charcoal	QC-500ug-a	27776	25667.84	-8%	-7%
Charcoal	QC-500ug-b	29168	27349.80	-6%	
Charcoal	QC-2000ug-a	129706	114626.17	-12%	-11%
Charcoal	QC-2000ug-b	129890	116884.64	-10%	

Event 9683E03d

Charcoal	QC-0ug-a	0			
Charcoal	QC-0ug-b	0			
Charcoal	QC-2000ug-a	129706	113754.40	-12%	-13%
Charcoal	QC-2000ug-b	129890	112775.40	-13%	

Rounding differences may occur due to electronic handling of numbers

Appendix B

Validated Methods for the Quantitation of Parent Dodecamethylcyclohexasiloxane in Blood, Feces and Expired Volatiles

Procedure for Determination of D_6 in Biological Matrices

(Blood and Feces)

PROCEDURE FOR DETERMINATION OF D_6 IN BIOLOGICAL MATRICES (BLOOD and FECES)

PURPOSE

To describe a method for extraction and quantification of Dodecamethylcyclohexasiloxane (D_6) in biological matrices.

EQUIPMENT

1. Chemicals
   a. Dodecamethylcyclohexasiloxane (D_6) — supplied by DCC
   b. Tetrakis(trimethylsiloxy)silane (M_4Q) — supplied by Gelest
   c. Tetrahydrofuran anhydrous 99.9% (THF) — supplied by Aldrich
   d. Magnesium sulfate ($MgSO_4$) — supplied by Fisher

2. Equipment

GC/MS	HP 6890 HP Chemstation Software	Hewlett Packard
Column	HP-5 MS 30m x 0.25mm ID, 0.25µm film thickness	Hewlett Packard
Merlin Microseal	Microseal-221 nut Microseal-H septum	Merlin Instrument Company
Centrifuge		Beckman GS-6R
Vortexer		VWR Multi-Tube Vortexer
Autosampler vials	2 mL crimp top, clear glass	Hewlett Packard
Limited volume inserts	100µL glass	Alltech
Aluminum crimp caps	teflon-lined red/orange butyl rubber septa	Hewlett Packard
Extractant collection vials and caps	glass – PTFE lined	Alltech
Round bottom vial	1.7 mL crimp top, clear glass	Alltech
scissors	stainless steel surgical	
glass beads	4mm glass	Fisher

Note: equivalent equipment may be substituted for any of the above.

3. General

An analytical balance shall be used for gravimetric preparation of all standards and samples on a weight of solute per weight of solution basis.

PREPARATION OF REAGENTS

1. Internal Standard Stock Preparation

Accurately weigh and record to the nearest 0.1mg in a glass vial previously capped and tared, approximately 20.0 mg of M_4Q. Add approximately 50 mL of THF, obtain final weight of solution and mix well. (Conc. approximately 450000 ng/g).

2. Internal Standard Working Solution

The internal standard working solution (ISTD) which is added to all working solvent standards and QC matrix samples consists of THF containing M_4Q (IS). To prepare 100 mL of internal standard solution, weigh and record to the nearest 0.1 mg, approximately 3 mL of internal standard stock solution in a suitable glass vial. Add approximately 100 mL of THF, cap and vortex gently for 30 seconds. Obtain the final weight of the standard solution. (Conc. approximately 13500 ng/g).

3. Standard Preparation (STD)

3.1 STD STK

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg, approximately 35.0 mg of D_6. Add approximately 10 mL of THF, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and THF. (Conc. approximately 4000000 ng/g).

3.2 STD STK 1

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg approximately 0.3 mL of STD STK (above). Add approximately 10 mL of THF, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and THF. (Conc. approximately 120000 ng/g)

3.3 STD STK 2

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg approximately 0.16 mL of STD STK 1 (above). Add approximately 10 mL of THF, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and THF. (Conc. approximately 1920 ng/g)

4. QC Stock Solution Preparation

4.1 QC STK

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg, approximately 35.0 mg of D_6. Add approximately 10 mL of THF, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and THF. (Conc. approximately 4000000 ng/g).

4.2 QC STK 1

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg approximately 0.5 mL of QC STK (above). Add approximately 10 mL of THF, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and THF. (Conc. approximately 197000 ng/g)

4.3 QC STK 2

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg approximately 0.4 mL of QC STK 1(above). Add approximately 10 mL of THF, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and THF. (Conc. approximately 7870 ng/g).

NOTE:
Record all STD STK and QC STK preparation information on an appropriate form.
Reagent volumes and weights may be scaled up or down proportionally.

STD CURVE PREPARATION

Place approximately 250 mg of $MgSO_4$ into round bottom glass vials. Prepare solvent standards with the appropriate standard stock solution and internal standard solution according to the following table. Obtain the weight of the aliquot of standard stock and the aliquot of internal standard solution placed in the vial containing $MgSO_4$ all to the nearest 0.1mg. Vortex each standard approximately 15 seconds. Dry (with $MgSO_4$) at least 1 hour. Centrifuge each standard at a setting of 2800 rpm for approximately 15 min. Place an aliquot of each standard in a limited volume insert in an autosampler vial and analyze by GC-MS. It may not be necessary to prepare all standards as long as the samples are covered.

Table 1: Standards

Standard ID	Volume of STD STK (mL)	STD STK used	Volume of Internal standard solution (mL)	Total Volume (mL)	Approximate amount of IS added (ng)	Approximate amount D_6 added (ng)
Blank	0	none	0.5	1.00	6750	0.00
STD 20	0.010	STD STK 2	0.5	1.00	6750	19.2
STD 50	0.025	STD STK 2	0.5	1.00	6750	48.0
STD 100	0.050	STD STK 2	0.5	1.00	6750	96.0
STD 300	0.150	STD STK 2	0.5	1.00	6750	288.0
STD 600	0.300	STD STK 2	0.5	1.00	6750	576.0
STD 1000	0.500	STD STK 2	0.5	1.00	6750	960.0
STD 2500	0.020	STD STK 1	0.5	1.00	6750	2400.0
STD 6000	0.050	STD STK 1	0.5	1.00	6750	6000.0
STD 12000	0.100	STD STK 1	0.5	1.00	6750	12000.0
STD 25000	0.200	STD STK 1	0.5	1.00	6750	24000.0
STD 50000	0.400	STD STK 1	0.5	1.00	6750	48000.0
STD 80000	0.020	STD STK	0.5	1.00	6750	80000.0
STD 140000	0.035	STD STK	0.5	1.00	6750	140000.0
STD 220000	0.055	STD STK	0.5	1.00	6750	220000.0
STD 400000	0.100	STD STK	0.5	1.00	6750	400000.0

STD 800000	0.200	STD STK	0.5	1.00	6750	800000.0
STD 1200000	0.300	STD STK	0.5	1.00	6750	1200000.0
STD 1600000	0.400	STD STK	0.5	1.00	6750	1600000.0
STD 2000000	0.500	STD STK	0.5	1.00	6750	2000000.0

STD (ng) = Conc. STD STK (1 or 2) (ng/g) X Wt. of STD (g)

Standard stock solutions and working standard solutions are stable at room temperature (18 ± 8°C) for up to 14 days.

QC SAMPLE PREPARATION

Prepare each matrix spike in duplicate according to the table below. Place approximately 150 mg of Blood or 250 mg feces homogenate) into the appropriate vials and spike with appropriate QC stock solution as shown below. For blood QC's only QC-0 through QC-3 are needed. Volumes are given for comparison, but obtain weights of all aliquots for calculation purposes (to the nearest 0.1mg).

Table 2: QC Matrix Samples (blood and Feces homogenates)

Standard ID	Volume of STD STK (mL)	STD STK used	Volume of Internal standard solution (mL) (1st extract)	Total Volume[a] (mL)	Approximate amount of IS added (ng)	Approximate amount D_6 added (ng)
QC-0	0.000	none	0.5	1.00	6750	0
QC-1	0.005	QC STK 2	0.5	1.00	6750	39
QC-2	0.005	QC STK 1	0.5	1.00	6750	985
QC-3	0.025	QC STK 1	0.5	1.00	6750	4925
QC-4	0.025	QC STK	0.5	1.00	6750	98400

[a] Total volume is based on 1 extraction at approximately 0.5 mL of internal standard solution (weight obtained) and an additional extraction at approximately 0.5 mL of THF.

QC samples are then extracted with THF according to the Sample Preparation Section below starting with step 1.2 (don't add blood in step 1.3) or 2.4 depending on the matrix used.

SAMPLE PREPARATION

1. Blood Extraction

   1.1 Add ~5 glass beads to extraction vial.

   1.2 Add ~0.5 mL of internal standard solution and obtain weight.

   1.3 Add approximately 150-250 mg of Blood; obtain weight, and vortex at least 5 minutes.

   1.4 Centrifuge at least 5 minutes at 2800 rpm and transfer the supernatant to a new pre-weighed glass vial.

   1.5 Add 0.5mL of THF to the blood vial for a second extraction and vortex at least 5 minutes.

1.6 Centrifuge at least 5 minutes at 2800 rpm and transfer the supernatant to the same glass vial containing the first extract. Obtain weight of combined extracts. (note: extractant weight only needed if extractant will be used for Radiochemical analysis.)

1.7 Add approximately 250 mg of $MgSO_4$ to glass round bottom vials. Transfer ~600uL of each of the above extracts to the glass round bottom vials, cap and vortex for approximately 15 seconds, and allow to dry at least 1 hour.

1.8 Centrifuge the samples for at least 15 minutes at 2800 rpm.

1.9 Transfer an aliquot of the supernatant to a low volume insert in a GC autosampler vial.

2 Feces or GI Content Homogenate Extraction

2.1 Obtain jar containing feces homogenate sample from freezer and allow to thaw on ice.

2.2 Vortex gently by hand.

2.3 Accurately weigh and record to the nearest 0.1 mg in an appropriate vial, approximately 0.25 g of feces homogenate. Place original container back in -80°C storage. Place 0.25 g aliquot vial on ice.

2.4 Remove from ice, wipe water from outside of vial and obtain tare weight of vial (or tare balance) containing the homogenate sample. Add 0.5 mL of internal standard solution, obtain weight and vortex for at least 5 minutes.

2.5 Sonicate sample for at least 5 minutes.

2.6 Centrifuge at least 5 minutes at approximately 2800rpm (until supernatent separates) and transfer the supernatant to a new pre-weighed glass vial.

2.7 Add 0.5 mL of THF to the vial containing the feces homogenate for a second extraction and vortex for at least 5 minutes.

2.8 Sonicate sample for at least 5 minutes.

2.9 Centrifuge at least 5 minutes at approximately 2800rpm (until supernatent separates) and transfer the supernatent to the vial containing the 1st extract.

2.10 Repeat steps 2.7 to 2.9 one more time and combine the supernatent with the 1st two extracts. Obtain the final weight of the vial containing the combined 3 extracts. (note: extractant weight only needed if extractant will be used for Radiochemical analysis.)

2.11 Add approximately 250 mg of $MgSO_4$ to glass round bottom vials. Transfer ~600uL of each of the above extracts to the glass round bottom vials, cap and vortex for approximately 15 seconds, and allow to dry at least 1 hour.

2.12 Centrifuge the samples for at least 15 minutes at approximately 2800 rpm.

2.13 Transfer an aliquot of the supernatant to a low volume insert in a GC autosampler vial.

Note: Once samples are in extraction solvent, samples are stable up to 14 days at -20±4 degrees C.

ANALYSIS

Samples shall be analyzed by GC/MS using the instrument parameters shown in Table 3.

Table 3. Analysis Parameters

Instrument:	Hewlett Packard 6890 Gas Chromatograph/Mass Selective Detector		
Column:	Hewlett Packard HP-5MS 30m x 0.25mm ID with 0.25µm film thickness		
Carrier Gas:	Helium, initial pressure 9.29 psi, 1.0 mL/min, constant flow on		
Injection:	temperature 250°C, splitless, purge time 0.00min, 2µL injection		
Oven Ramp:	Initial 70°C for 3.0 min, ramp to 250°C at 30°C/min, hold for 1 min, total run time 13.57 min		
Detection:	MSD transfer line temperature 280°C		
	Quantitation ions:	D_6:	429 m/z at 100msec
		M_4Q:	281 m/z at 100msec

Single injection analysis of each sample is sufficient.

Sample Analysis Order (Example)
Analyze each matrix in separate analysis runs. Separate analysis runs may occur on the same day.

Solvent Blank
Solvent Internal Standard Blank (3 injections)
Solvent Calibration Standards, (Low to High)
Solvent Blank
QC Samples (Low to High)
Solvent Blank
Solvent Standard
Solvent Blank
10 Samples or Less
Solvent Standard
Solvent Blank

Repeat this Solvent standard and Sample analysis pattern until all samples are analyzed.

DATA ANALYSIS

This section describes the calculations for the calibration of the GC/MS and the method to determine the amount of D_6 per gram of blood or feces.

All calculations for routine sample analysis shall be performed using a Microsoft Excel spreadsheet (a spreadsheet which has been prepared for a specific application and has been confirmed by an independent review to perform calculations as defined; subsequent uses of the spreadsheet require 100% check of all entered data). Non-routine calculations shall be prepared and reviewed as directed by the Study Director (or designee).

1. Instrument Calibration Calculations

Calibration of the mass spectrometer is performed using D_6 concentrations expressed in terms of ng D_6. The nominal concentrations of calibration standards are shown in Table 1. The standard curve may be split into up to 4 ranges depending on the range of the instrument as long as at least 4 standards make up a range.

D_6 Calibration Equation

Calculate a linear equation, using a suitable linear regression program, for D_6 where x = concentration (ng D_6) and y = peak area response ratio for the calibration standards from the GC/MS analysis. Enter the resulting slope (m) and y-intercept (b) from each equation into the spreadsheet.

$y = mx + b$, where y = peak area response ratio and x = ng D_6

2. Calculation of D_6 Concentrations in Samples (ng D_6)

The concentration of D_6 in a sample extract is calculated once the slope (m) and y-intercept (b) have been entered into the spreadsheet. The concentration (ng D_6) of D_6 in a sample extract is calculated by substitution of the peak area response ratio for y into the linear equation generated from calibration standards and solving for x, $(x = (y-b)/m)$.

3. Calculation of D_6 Concentrations in Samples (μg D_6/g matrix)

Calculation of D_6 concentration (μg/g) in sample matrix is as follows:

D_6 (μg/g) = D_6(ng) / Sample matrix weight (g) X 1μg/1000ng

If the sample is homogenized with water (saline) prior to extraction for example liver, lung or feces/GI content, the final concentration D_6 in the matrix is calculated as follows:

D_6 (μg/g) = D_6 (μg/g homogenate) X total homogenate weight (g) / matrix weight (g)

Note: An assumption is made that the amount of internal standard added (~0.5 mL) is the same for all samples and standards. If when obtaining the weights of these additions, it is found that the weights vary significantly (%CV > 5%), or at the discretion of the bioanalytical supervisor, then all calculations will have to be adjusted to correct for the differing amounts of internal standard added. For example, the calibration curve would be generated with x = ng D_6/ng IS. Each sample would be calculated according to the calibration curve generated by solving for x (ng D_6/ng IS). The amount of D_6 (ng) in each sample is then calculated by multiplying the ratio found (ng D_6/ng IS) by the ng IS added. The subsequent calculations for determining ug D_6/g sample would be the same as described above.

DATA ACCEPTANCE

1. Calibration Acceptance Criteria

   Agreement between the analyzed and prepared concentrations of D_6 in the calibration standards must be achieved to prove conformance to the linear calibration model. The percent relative error, calculated by the qualified spreadsheet, shall be used to prove conformance and is calculated by subtracting the analyzed concentration from the prepared concentration, and then dividing by the prepared concentration and multiplying by 100. The percent relative error shall be within 15% for every calibration standard analyzed for the calibration to be acceptable. Calibrations that do not meet these requirements shall be brought to the attention of the Study Director (or designee). Exceptions to this calibration acceptance criteria shall be made if all samples are bracketed by calibration standards that did meet the calibration acceptance criteria. The solvent standards that are run intermittently throughout the run are to be within 15% for the run to be accepted. Any samples run before a standard meeting this acceptance criteria will be accepted. If any standards do not meet this acceptance criteria, any samples run after the standard will be evaluated by the bioanalytical supervisor if they will be accepted.

REPORTING AND DATA COMPLETION

The chemistry technician shall be responsible for submission of a completed data packet to the Study Director (or designate). This data packet shall include, as a minimum:

1. Hard copies of GC/MS data (including instrument parameters and sequence)
2. The calibration curve and the data from which it was generated
3. Data reduction spreadsheet

The chemistry technician shall also be responsible for completion of the notebook:

1. Calibration Standard Form attached in notebook and completed
2. QC Sample Preparation Form attached in notebook and completed
3. One page describing which samples were analyzed and any other comments on sample workup and analysis

QUALITY CONTROL

The chemistry technician shall check the data packet for accuracy and completeness prior to forwarding to the Study Director (or designate). The Study Director (or designate) shall provide a one-over-one check for accuracy and completeness and ensure that all GLP record keeping practices were correctly performed.

Procedure for Determination of D_6 in Expired Volatiles

(Charcoal Tubes)

PROCEDURE FOR DETERMINATION OF D_6 EXPIRED VOLATILES (CHARCOAL TUBES)

PURPOSE

To describe a method for extraction and quantification of Dodecamethylcyclohexasiloxane (D_6) in expired volatiles that have been trapped on charcoal tubes.

EQUIPMENT

1. Chemicals
   a. Dodecamethylcyclohexasiloxane (D_6) — supplied by DCC
   b. Tetrakis(trimethylsiloxy)silane (M_4Q) — supplied by Gelest
   c. Toluene 99.9% — supplied by Fisher
   d. Magnesium sulfate ($MgSO_4$) — supplied by Fisher

2. Equipment

GC/MS	HP 6890 HP Chemstation Software	Hewlett Packard
Column	HP-5 MS 30m x 0.25mm ID, 0.25µm film thickness	Hewlett Packard
Merlin Microseal	Microseal-221 nut Microseal-H septum	Merlin Instrument Company
Centrifuge		Beckman GS-6R
Vortexer		VWR Multi-Tube Vortexer
Autosampler vials	2 mL crimp top, clear glass	Hewlett Packard
Limited volume inserts	100µL glass	Alltech
Aluminum crimp caps	teflon-lined red/orange butyl rubber septa	Hewlett Packard
Desorption vials vials and caps	20 mL glass – aluminum lined	Fisher
Round bottom vial	1.7 mL crimp top, clear glass	Alltech
Small file or glass scoring tool		Fisher
Tweezers		Fisher

Note: equivalent equipment may be substituted for any of the above.

3. General

An analytical balance shall be used for gravimetric preparation of all standards and samples on a weight of solute per weight of solution basis.

PREPARATION OF REAGENTS

1.1 Internal Standard Stock A Preparation

Accurately weigh and record to the nearest 0.1mg in a glass vial previously capped and tared, approximately 50.0 mg of M_4Q. Add approximately 12.5 mL of toluene, cap and vortex gently for 30 seconds. Obtain the final weight of the standard solution. (Conc. approximately 4000000 ng/g).

1.2 Internal Standard Stock B Preparation

Accurately weigh and record to the nearest 0.1mg in a glass vial previously capped and tared, approximately 200 mg of Internal Standard Stock A (above). Add approximately 50 mL of toluene, cap and vortex gently for 30 seconds. Obtain the final weight of the standard solution. (Conc. approximately 16000 ng/g).

1.3 Internal Standard Working Solution (toluene/ISTD)

The internal standard working solution (toluene/ISTD) which is used as the dilution/extraction solvent for all working solvent standards, QC matrix samples and study samples, consists of toluene containing M_4Q (IS). To prepare 4L of internal standard working solution, remove 40 mL of toluene from a 4L bottle of toluene and using a 10mL pipette, transfer 40 mL of Internal Standard Stock B (above) into the 4L bottle of toluene. Mix thoroughly and clearly identify the bottle with the new solution. (Conc. approximately 256 ng/g).

3. Standard Preparation (STD)

3.1 STD STK A

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg, approximately 50.0 mg of D_6. Add approximately 12.5 mL of toluene/ISTD, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and toluene/ISTD. (Conc. approximately 4000000 ng/g).

3.2 STD STK B

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg approximately 0.05mL of STD STK A (above). Add approximately 12.5 mL of toluene/ISTD, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and toluene/ISTD. (Conc. approximately 16000 ng/g)

3.3 STD STK 1000

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg approximately 0.63mL of STD STK B (above). Add approximately 10 mL of toluene/ISTD, cap and

vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and toluene/ISTD. (Conc. approximately 1000 ng/g)

3.4 STD STK 100

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg approximately 1.0mL of STD STK 1000 (above). Add approximately 10 mL of toluene/ISTD, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and toluene/ISTD. (Conc. approximately 100 ng/g)

4. QC Stock Solution Preparation

4.1 QC STK 200

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg, approximately 150.0 mg of D_6. Add approximately 0.75 mL of toluene, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and toluene. (Conc. approximately 200mg/g).

4.2 QC STK 50

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg approximately 0.25 mL of QC STK 200 (above). Add approximately 1 mL of toluene, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and toluene. (Conc. approximately 50mg/g)

4.3 QC STK 5

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg approximately 0.1mL of QC STK 50 (above). Add approximately 1 mL of toluene, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and toluene. (Conc. approximately 5mg/g).

4.4 QC STK 0.2

In a suitable glass vial, previously capped and tared, weigh and record to the nearest 0.1 mg approximately 0.04 mL of QC STK 5 (above). Add approximately 1 mL of toluene, cap and vortex gently for 30 seconds at motor speed 2. Obtain the final weight of standard and toluene. (Conc. approximately 0.2mg/g).

NOTE:
Record all STD STK and QC STK preparation information on an appropriate form.
Reagent volumes and weights may be scaled up or down proportionally.

STD CURVE PREPARATION

Place approximately 250 mg of $MgSO_4$ into round bottom glass vials. Prepare solvent standards with the appropriate standard stock solution and toluene/ISTD solution according to the following table. Obtain the weight of the aliquot of standard stock and the final solution weight placed in the vial containing

$MgSO_4$ all to the nearest 0.1mg. Vortex each standard approximately 15 seconds. Dry (with $MgSO_4$) at least 1 hour. Centrifuge each standard at a setting of 2800 rpm for approximately 15 min. Place an aliquot of each standard in a limited volume insert in an autosampler vial and analyze by GC-MS.

Table 1: Solvent Standards

Standard ID	Volume of STD STK (µL)	STD STK used	Volume of toluene/ISTD (µL)	Total Volume (mL)	Approximate Conc. of D_6 added (ng/g)
Blank	0	none	1000	1	0
20	200	STD STK 100	800	1	20
50	500	STD STK 100	500	1	50
100	1000	STD STK 100	0	1	100
200	200	STD STK 1000	800	1	200
400	400	STD STK 1000	600	1	400
600	600	STD STK 1000	400	1	600
800	800	STD STK 1000	200	1	800
1000	1000	STD STK 1000	0	1	1000
2000	126	STD STK B	874	1	2016
4000	252	STD STK B	748	1	4032
6000	375	STD STK B	625	1	6000
8000	500	STD STK B	500	1	8000
10000	630	STD STK B	370	1	10080
12000	750	STD STK B	250	1	12000
Stock B	1000	STD STK B	0	1	16000
120000	30	STD STK A	970	1	120000
200000	50	STD STK A	950	1	200000
400000	100	STD STK A	900	1	400000

STD (ng/g) = Conc. STD STK used (ng/g) X Wt. of STD (g) / Total ~ weight (g)

Standard stock solutions and working standard solutions are stable at 4±4°C for up to 21 days.

QC SAMPLE PREPARATION

Prepare each matrix spike in duplicate according to the table below. Score the end of a blank charcoal tube and break the tip and remove. Fill a syringe to the mark indicated in the table below and weigh the syringe full. Spike the blank charcoal tube by placing the tip of the syringe into the level of charcoal. Weigh the empty syringe and by difference determine the weight of the spike.

Table 2: QC Charcoal Tube Samples

QC Sample ID	Volume of QC STK (uL)	QC STK used	Volume of toluene/ISTD for extraction (mL)	Total Volume (mL)	Approximate Conc. of D_6 added (ng/g)
0ug	0	none	15	15	0
2ug	10	QC STK 0.2	15	15.01	133
50ug	10	QC STK 5	15	15.01	3333
500ug	10	QC STK 50	15	15.01	33333
2000ug	10	QC STK 200	15	15.01	133333

QC charcoal tube samples are then extracted with toluene/ISTD according to the Sample Preparation Section below starting with step 1.b.

SAMPLE PREPARATION

1. Charcoal Tube Extraction

a. Remove charcoal tube samples from –20°C frozen storage and allow to warm to 4±4°C in the walk-in refrigerator.
b. Processing of the charcoal tubes should be done in the walk-in refrigerator using chilled toluene/ISTD [a]. ([a] Due to exothermic reaction caused by the addition of the charcoal to the toluene)
c. Record information on the appropriate form.
d. Score the end of the charcoal tube containing the cotton plug with a small file or glass scoring tool. Score it back from the end far enough to allow easy access to the cotton plug with tweezers.
e. Break off the end of the charcoal tube.
f. Crack the tube and deliver entire contents to a 20 ml scintillation vial containing ~15 ml of pre-weighed appropriate solvent (e.g. toluene/ISTD).
g. Wrap the caps of the vials with Teflon tape to prevent evaporation of solvent.
h. Allow solvent to desorb analytes from charcoal for at least 24hours.

Radioactivity Analysis

1) Label caps of LSC vials.
2) Add approximately 5 ml of the scintillation cocktail to each of two 7 ml scintillation vials. If 20 ml scintillation vials are used, add approximately 15 ml of scintillation cocktail.
3) Remove two 100 - 500 µl aliquots of solvent from the vial containing the charcoal and transfer them to the two scintillation vials.
4) Record the weights of the aliquots.
5) Mix gently, and place vials in a scintillation counter tray for radioactivity analysis.

GC/MS Analysis

1) Place approximately 250mg of $MgSO_4$ in round bottom vials.
2) Transfer approximately 600µL aliquots of the solvent above each charcoal sample to the vial containing $MgSO_4$ and allow to dry for at least 1 hour.
3) Centrifuge each of the above dried samples at a setting of 2800 rpm for approximately 15 min.
4) Place an aliquot of each standard in a limited volume insert in an autosampler vial and analyze by GC-MS.

Note: Expired volatile tubes are stable at -20±4 degrees C for 15 days. Once samples are in extraction solvent, samples are stable up to 21 days at 4±4 degrees C.

ANALYSIS

Samples shall be analyzed by GC/MS using the instrument parameters shown in Table 3.

Table 3. Analysis Parameters

Instrument:	Hewlett Packard 6890 Gas Chromatograph/Mass Selective Detector		
Column:	Hewlett Packard HP-5MS 30m x 0.25mm ID with 0.25um film thickness		
Carrier Gas:	Helium, initial pressure 10.5 psi, 1.0 mL/min, constant flow on		
Injection:	temperature 250°C, splitless, purge time 0.00min, 2μL injection		
Oven Ramp:	Initial 100°C for 3.0 min, ramp to 250°C at 25°C/min, hold for 1min, total run time 10.00 min		
Detection:	MSD transfer line temperature 280°C		
	Quantitation ions:	D_6:	429 m/z at 100msec
		M_4Q:	281 m/z at 100msec

Single injection analysis of each sample is sufficient.

Sample Analysis Order (Example)
Analyze each matrix in separate analysis runs. Separate analysis runs may occur on the same day.

Solvent Blank
Solvent Internal Standard Blank (3 injections)
Solvent Calibration Standards, (Low to High)
Solvent Blank
QC Samples (Low to High)
Solvent Blank
Solvent Standard
Sample Blank
10 Samples or Less
Solvent Standard
Sample Blank

Repeat this Solvent standard and Sample analysis pattern until all samples are analyzed.

DATA ANALYSIS

This section describes the calculations for the calibration of the GC/MS and the method to determine the amount of D_6 in each charcoal tube.

All calculations for routine sample analysis shall be performed using a Microsoft Excel spreadsheet (a spreadsheet which has been prepared for a specific application and has been confirmed by an independent review to perform calculations as defined; subsequent uses of the spreadsheet require 100% check of all entered data). Non-routine calculations shall be prepared and reviewed as directed by the Study Director (or designee).

1. Instrument Calibration Calculations

   Calibration of the mass spectrometer is performed using D_6 concentrations expressed in terms of ng D_6/g toluene. The nominal concentrations of calibration standards are shown in Table 1. The standard curve may be split into up to 4 ranges depending on the range of the instrument as long as at least 4 standards make up a range.

D_6 Calibration Equation

Calculate a linear equation, using a suitable linear regression program, for D_6 where x = concentration (ng D_6/g) and y = peak area response ratio for the calibration standards from the GC/MS analysis. Enter the resulting slope (m) and y-intercept (b) from each equation into the spreadsheet.

y = mx + b, where y = peak area response ratio and x = ng D_6/g

2. Calculation of D_6 Concentrations in Samples (µg D_6)

The concentration of D_6 in a sample extract is calculated once the slope (m) and y-intercept (b) have been entered into the spreadsheet. The concentration (ng D_6/g toluene) of D_6 in a sample extract is calculated by substitution of the peak area response ratio for y into the linear equation generated from calibration standards and solving for x, (x = (y-b)/m).

3. Calculation of D_6 Concentrations in Samples (µg D_6)
Calculation of D_6 concentration (µg) in sample matrix is as follows:

D_6 (µg) = D_6(ng)/g toluene X g toluene/ISTD used for extraction X 1µg/1000ng

DATA ACCEPTANCE

1. Calibration Acceptance Criteria

Agreement between the analyzed and prepared concentrations of D_6 in the calibration standards must be achieved to prove conformance to the linear calibration model. The percent relative error, calculated by the qualified spreadsheet, shall be used to prove conformance and is calculated by subtracting the analyzed concentration from the prepared concentration, and then dividing by the prepared concentration and multiplying by 100. The percent relative error shall be within 15% for every calibration standard analyzed for the calibration to be acceptable. Calibrations that do not meet these requirements shall be brought to the attention of the Study Director (or designee). Exceptions to this calibration acceptance criteria shall be made if all samples are bracketed by calibration standards that did meet the calibration acceptance criteria. The solvent standards that are run intermittently throughout the run are to be within 15% for the run to be accepted. Any samples run before a standard meeting this acceptance criteria will be accepted. If any standards do not meet this acceptance criteria, any samples run after the standard will be evaluated by the bioanalytical supervisor if they will be accepted.

REPORTING AND DATA COMPLETION

The chemistry technician shall be responsible for submission of a completed data packet to the Study Director (or designate). This data packet shall include, as a minimum:

1. Hard copies of GC/MS data (including instrument parameters and sequence)
2. The calibration curve and the data from which it was generated
3. Data reduction spreadsheet

The chemistry technician shall also be responsible for completion of the notebook:

1. Calibration Standard Form attached in notebook and completed
2. QC Sample Preparation Form attached in notebook and completed
3. One page describing which samples were analyzed and any other comments on sample workup and analysis

QUALITY CONTROL

The chemistry technician shall check the data packet for accuracy and completeness prior to forwarding to the Study Director (or designate). The Study Director (or designate) shall provide a one-over-one check for accuracy and completeness and ensure that all GLP record keeping practices were correctly performed.

APPENDIX C

Statistical Analysis

To: Marina Jovanovic
Subject: Contributing scientist report: Statistical analysis of the data from study No. 9683

Submitted by: Trevor Newhook, biostatistician specialist on October 29, 2002

Signed for Trevor Newhook Roy A Campbell 02-Feb-2004
Roy A. Campbell Date
Manager, HES Operations
Health and Environmental Sciences

Summary

The objectives of this study evaluate absorption of ^{14}C-dodecamethylcyclohexasiloxane (^{14}C-D_6) through the gastrointestinal (GI) tract and to determine if there were any differences in absorption or distribution between males and females when ^{14}C-D_6 was delivered in corn oil. A dose of 1000 mg of D_6/kg of body weight was administered by oral gavage.

In blood, the radioactivity area under the curve (AUC) was significantly larger than the parent area under the curve for males and females, (225.93 vs. 108.36) for males and (293.53 vs. 177.20) for females. The mean parent area under the curve in blood was significantly larger for females compared to males, (177.20 vs. 108.36). The radioactivity area under the curve in blood was also significantly larger for females compared to males (293.53 vs. 225.93). There was no significant difference in the metabolite areas under the curves ($AUC_{radioactivity} - AUC_{parent}$) in blood between females and males (116.33 vs. 117.57).

In feces, the radioactivity area under the curve did not significantly differ from the parent area under the curve and there was no significant difference in the radioactivity areas under the curves between sexes.

In charcoal, the radioactivity area under the curve did not significantly differ from the parent area under the curve and there was no significant difference in the radioactivity areas under the curves between sexes.

When comparing the mean % level of endpoints of the administered dose, there were significant differences between the mean % level of administered dose between the sexes in CO_2 (higher in males), adrenals and gastrointestinal tract (higher in females).

Introduction

The objectives of this study was to evaluate absorption of ^{14}C-dodecamethylcyclohexasiloxane (D_6) through the gastrointestinal (GI) tract and to determine if there were any differences in absorption or distribution between males and females when D_6 was delivered in corn oil.

Methods

The responses in each analysis were D6 parent content or percent of administered radioactivity recovered in a given sample. All statistical analyses were carried out using SAS®, version 8.2. The probability of Type I error (α) was 5 %. Comparisons were made using areas under the curves (AUC). The AUCs were calculated for parent and radioactive content and the areas were compared between males and females. Differences in the AUCs between sexes were determined by constructing a 95% confidence interval for the difference ($AUC_{male} - AUC_{female}$) using the method of Nedelman and Jia (1998). Confidence intervals for the areas under the curves were constructed using the method of Nedelman, Gibansky and Lau (1995). If the confidence interval did not contain the value zero, then the mean AUCs were considered to be statistically significant. The mean % levels of all endpoints of the administered dose were analyzed using Analysis of Variance if the data were normally distributed with homogeneous variances, otherwise Wilcoxon's test was used to analyze the data. No further multiple comparison tests were used following the ANOVA or Wilcoxon test since only the means of only two groups were being compared.

Results

Blood

Parent vs. Radioactivity AUC Comparison (Table 1)

The radioactivity area under the curve was significantly larger than the parent area under the curve in blood in both females (293.53 vs. 177.20, $p=2.9698 \times 10^{-8}$) and males (225.93 vs. 108.36, p=0).

AUC Comparison between Sexes (Table 1)

The parent area under the curve in blood was significant larger for females compared to males, 177.20 vs. 108.36, p=0.000019767) and the radioactivity area under the curve was significantly larger in females compared to males in blood, (293.53 vs. 225.93, p=0.000002738).

Metabolite AUC Comparison between Sexes (Table 1)

The metabolite area under the curve ($AUC_{radioactivity} - AUC_{parent}$) in blood was not significantly different for females compared to males (116.33 vs. 117.57, p=0.22124).

Feces

Parent vs. Radioactivity AUC Comparison (Table 2)

There was no significant difference between the parent area under the curve in feces and radioactivity area under the curve in females (596859.84 vs. 664925.90, p=0.11454) or males (695573.24 vs. 696291.26, p=0.24854).

AUC Comparison between Sexes (Table 2)

There was no significant difference in the radioactivity areas under the curves in feces between females and males (664925.90 vs. 696291.26, p=0.18670). There was no significant difference in the parent areas under the curves in feces between females and males, (596859.84 vs. 695573.24, p=0.075025).

Charcoal

Parent vs. Radioactivity AUC Comparison (Table 3)

There was no significant difference between the parent area under the curve in charcoal and radioactivity area under the curve when D_6 was administered in corn oil to females (17887.77 vs. 19101.98, p=0.20805) or to males (23759.53 vs. 24730.65, p=0.20471).

AUC Comparison between Sexes (Table 3)

There was no significant difference in the parent areas under the curves between males and females (23759.53 vs. 17887.77, p=0.058556), and there was no significant difference in the radioactivity areas under the curves between males and females (24730.65 vs. 19101.98, p=0.075275).

Endpoints *(Table 4)*

When comparing mean endpoints between females and males, there was a significantly higher mean level of % dose recovered in adrenals, (0.000632 vs. 0.000170, p=0.0304) and gastrointestinal tract (0.00664 vs. 0.00515, p=0.0330) in females and a higher % dose recovered in CO_2 in males (0.125 vs. 0.093, p=0.0304).

Half-lives in blood

In males, under the parent curve, the half-life for $T_{1/2}$' phase was 8.55. Under the radiolabeled curve, the half-life for $T_{1/2}$' was 15.31 and 104.77 for $T_{1/2}$".

In females, under the parent curve, the half-life for $T_{1/2}$' was 18.93. Under the radiolabeled curve, the half-life for $T_{1/2}$' was 25.22 and 117.62 for $T_{1/2}$".

Note: $T_{1/2}$"for the parent curve male and females could not be determined because the concentrations for males 72 hour through 168 hour and females 96 hour through 168 hour were below limit of quantitation.

Tables

Table 1. Summary Table for Areas Under the Curves between Sexes in Blood

	Sex	Mean Area Under the Curve (µgXhr/g)	Standard Error
Parent	Female*+	177.198	12.6737
	Male*+	108.362	3.8071
Radioactivity	Female*+	293.531	11.3244
	Male*+	225.934	5.7452
Metabolite	Female*+	116.332	6.90938
	Male*+	117.572	4.94056

* - Statistically significant difference between sexes at α=0.05.
+ - Statistically significant difference between parent and radioactivity curves within sexes at α=0.05.

Table 2. Summary Table for Areas Under the Curves between Sexes in Feces

	Sex	Mean Area Under the Curve (µgXhr/g)	Standard Error
Parent	Female	596859.84	44845.95
	Male	695573.24	74837.96
Radioactivity	Female	664925.90	73399.84
	Male	696291.26	58467.71

Table 3. Summary Table for Areas Under the Curves between Sexes in Charcoal

	Sex	Mean Area Under the Curve (µgXhr/g)	Standard Error
Parent	Female	17887.77	3802.00
	Male	23759.53	2673.09
Radioactivity	Female	19101.98	4128.90
	Male	24730.65	3116.15

Table 4. Summary Statistics for Endpoints

	Variable	N	Mean (% of administered dose)	Std Dev	Std Error
Females					
Group 3	Urine	4	0.322057	0.046416	0.023208
	Expair	4	11.210770	9.570548	4.785274
	CO2 *	4	0.092957	0.020856	0.010428
	Carcass	4	0.164539	0.022411	0.011206
	Adrenal *	4	0.000632	0.000071	0.000036
	Lung	4	0.000676	0.000449	0.000224
	Ovaries	4	0.000443	0.000077	0.000038
	Fat	4	0.001098	0.000288	0.000144
	Spleen	4	0.000393	0.000094	0.000047
	Kidneys	4	0.002638	0.000386	0.000193
	Liver	4	0.030679	0.006353	0.003177
	GItract *	4	0.006641	0.000937	0.000469
	Tissues	4	0.043199	0.007655	0.003828
	Perdoseabs	4	11.833522	9.577656	4.788828
	Perdosefeces	4	82.789727	13.647448	6.823724
	Gitract2	4	0.003956	0.001411	0.000706
	Cagerinse	4	0.032495	0.033693	0.016847
	Perdoseexc	4	82.826173	13.620215	6.810107
	Perdosetiscar	4	0.207738	0.029916	0.014958
	Totalrec	4	94.659725	6.790619	3.395309
Males					
Group 4	Urine	4	0.377925	0.034775	0.017388
	Expair	4	11.197973	3.661037	1.830518
	CO2 *	4	0.124537	0.004168	0.002084
	Carcass	4	0.144362	0.012490	0.006245
	Adrenal *	4	0.000170	0.000021	0.000011
	Lung	4	0.000735	0.000084	0.000042
	Ovaries	4	0.001016	0.000142	0.000071
	Fat	4	0.001044	0.000406	0.000203
	Spleen	4	0.000386	0.000088	0.000044
	Kidneys	4	0.002056	0.000746	0.000373
	Liver	4	0.022357	0.003604	0.001802
	GItract *	4	0.005149	0.000541	0.000270
	Tissues	4	0.032913	0.005143	0.002572
	Perdoseabs	4	11.877710	3.685845	1.842923
	Perdosefeces	4	84.761106	10.430171	5.215085
	GItract2	4	0.002688	0.000964	0.000482
	Cagerinse	4	0.013373	0.004415	0.002207
	Perdoseexc	4	84.777283	10.427821	5.213910
	Perdosetiscar	4	0.177275	0.016129	0.008065
	Totalrec	4	96.654903	8.920519	4.460260

* - Statistically significant difference between sexes at α=0.05.
- Expair = Expired air
- Perdosefeces = Percentage dose in feces
- GItract2=GI tract contents.
- Perdoseexc = Percentage dose excreted (Feces + GI contents + Cage rinse).
- PerDoseabs = Percent dose absorbed.
- Perdosetiscar = Percentage dose recovered in tissues and carcass.
- Totalrec = Total Recovery

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Nedelman, J.R., Gibiansky, E., and Lau, D.T.W. Applying Biler's Method for AUC Confidence Intervals to Sparse Sampling. Pharmaceutical Research, 12: 124-128, 1995

SAS/Lab® Software: User's Guide. Version 6. First Edition. SAS Institute Inc. Cary, N.C.

SAS/Stat® User's Guide. Version 8. First Edition. Volume 2. SAS Institute Inc. Cary, N.C.

Documentation

A copy of the SAS® Log and the SAS® output for this analysis is filed with the raw data in the study file.

APPENDIX D

Whole-Body Autoradiography

To: Marina Jovanovic

Date: 2/2/2004

Re: Study 9683- Whole-Body Autoradiography

Submitted by: _____ 2/2/04

Jane M. Regan
Principle Technician
Date

Title *Disposition of ^{14}C-Dodecamethylcyclohexasiloxane (D6) following Single Oral Administration To Fischer 344 Rats*

Introduction

The objective of this portion of the study was to qualitatively evaluate the tissue distribution and absorption of ^{14}C-dodecamethylcyclohexasiloxane (D6) and its potential metabolites in Fischer 344 rats by whole-body autoradiography. The test article was suspended in corn oil as a carrier and delivered as a single exposure by oral gavage. The whole-body autoradiography (WBA) portion of this study was performed concurrently with the mass balance and blood kinetic analysis. Experimental design and specific information regarding the test system, test article, and dosing may be found in the study protocol and in the main body of the final report.

Methods

One animal from Groups 5 (females) and 6 (males) was sacrificed at selected time points (1, 4, 12, 24, 48, 96, and 168 hours) by CO_2 asphyxiation following a single exposure by oral gavage of ^{14}C-D6 suspended in corn oil. The dosing solution was prepared to deliver a targeted radioactivity of 25 µCi/100g body weight and a nominal dose of 1000 mg D6/ kg of body weight for each animal. Immediately following euthanasia, each animal was frozen in a hexane/dry ice mixture at approximately -75° C and stored at -80° C ± 10. The frozen carcasses were embedded in a 4% aqueous solution of carboxymethylcellulose (Sigma Chemical Company, St. Louis, MO) which, when frozen, supported the carcass for sectioning on the CryoMacrocut® microtome (Leica, Deerfield, IL) with temperature maintained at approximately -20 ± 5°C. Sagittal sections of approximately 40 microns in thickness were collected at various levels to include major organs and tissues of interest. Non-dehydrated sections were mounted on a cardboard support, covered with a layer of mylar, and exposed to Kodak BioMax MR® radiographic film (Eastman Kodak Company, Rochester, NY) for 2 and 4 weeks. One representative section from each level was dehydrated within the cryochamber for 48 to 72 hours and retained as a reference for comparison with the film. At the end of the exposure periods, films were developed on a Cordell™ MXR-14 automatic film processor (Cordell, Peabody, MA). The reported images were digitally acquired from film with a Hewlett-Packard ScanJet Pro (Palo Alto, CA) with output in grayscale at a resolution of 200 PPI (pixels per inch).

Results

Film exposure times at -80°C were determined empirically at two and four weeks after developing films from the first animal in Group 5. All original films were reviewed and evaluated visually for the intensity of radioactivity relative to background. It should be noted that at the earlier time points (1 through 24 hours) the high intensity of radioactivity in the gastrointestinal tract tended to obscure adjacent organs rendering difficulty in visualization.

One hour following dosing, radioactivity was distributed throughout the rats of both sexes (Figures 1 and 2). In the female rat the highest concentration of radioactivity was found in the contents of the stomach and small intestines. Moderate levels were found in the fat, brown fat, ethmoturbinates, and the ventral and anal surfaces of the skin. Low levels (slightly above the background of the film) were present in the bone marrow, liver, and blood. In the male the highest concentrations were also found in the contents of the stomach and small intestines, as well as the ethmoturbinates and hard palette. Moderate amounts were found in the skin surface, fat, and esophagus.

Four hours after dosing, both the female and male (Figures 3 and 4), the highest amount of radioactivity was observed in the contents of the gastrointestinal tract (stomach, small intestines, cecum and colon). Both sexes exhibited a moderate amount in the fat and on the surface (ventral) of the skin. In the female, there were low levels in the liver, brown fat, blood, and bone marrow. In the male, the lowest levels of radioactivity were observed in the liver and brown fat.

Twelve hours after dosing, the female (Figure 5) had the highest amounts of radioactivity in the contents of the cecum and colon, as well as a portion of the contents of the stomach. Moderate amounts were noted in the contents of the small intestine. Low levels were noted in the brown fat, bone marrow, liver, and ventral skin surface. The male (Figure 6) had high to moderate levels in the liver, contents of the cecum and small intestine, residual amount of stomach content, rectum, and skin surface. The lowest amount was found in the brown fat, adrenal cortex, and bone marrow.

Twenty-four hours post dose the highest concentrations in the female (Figure 7) were seen in the contents of the stomach, cecum, colon and in the small intestines. The female had the lowest levels in the liver, brown fat, bone marrow, adrenal cortex, esophagus, and hard palette. The highest concentrations in the male (Figure 8) were seen in the cecum, colon, and a residual amount of the stomach content. The male had lowest levels in the brown fat, liver, adrenal cortex, myocardium, and bone marrow.

Radioactivity content had decreased by the 48 hour time point. The female (Figure 9) had moderate levels in the adrenal cortex, residual amount on the wall of the stomach, and the contents of the cecum and colon. Lower amounts were observed in the brown fat, liver, and myocardium. The male had (Figure 10) moderate levels in the contents of the cecum and colon and low in the liver, brown fat, myocardium, and adrenal cortex.

At 96 hours, the female (Figure 11) had low amounts of radioactivity in the brown fat, liver, bone marrow, myocardium, and adrenal cortex. The male (Figure 12) had low levels in the brown fat.

At 168 hours, the last time point, both female and male (Figures 13 and 14) had moderate amounts of radioactivity in the brown fat. Both had low levels (slightly above background) in the liver, bone marrow, and myocardium.

Both sexes showed comparatively similar patterns of disposition at each time point (Figures 15 and 16) with decreasing intensity of radioactivity over time. At 96 and 168 hour time points the intensity of the radioactivity had decreased significantly.

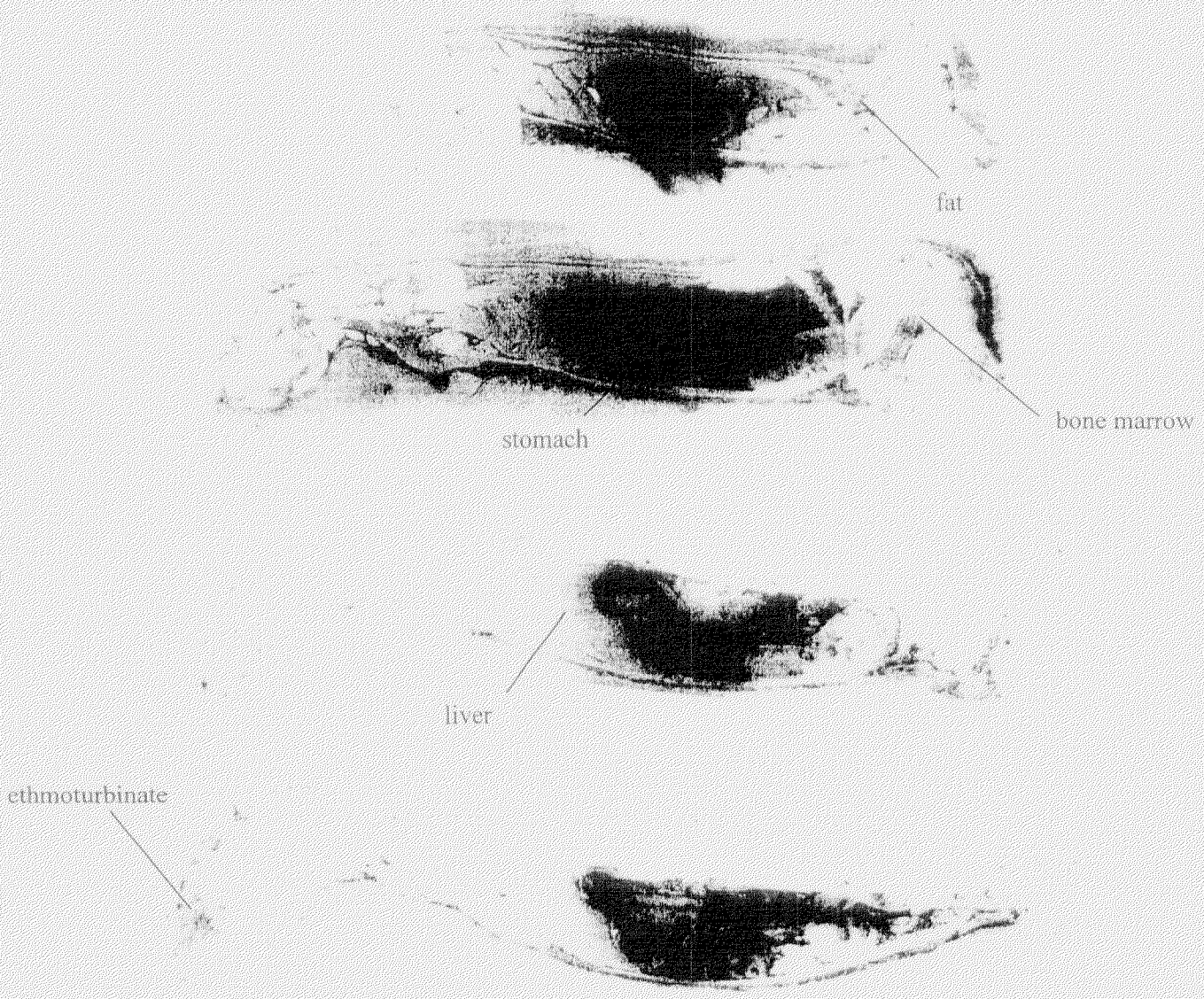
Records To Be Archived

Frozen carcasses, as well as the non-dehydrated sections will be disposed after authorization and finalization of the study. Films, dehydrated references sections, and processing records will be maintained in HES archives of Dow Corning Corporation, Midland, MI.

List of Figures

Figure 1

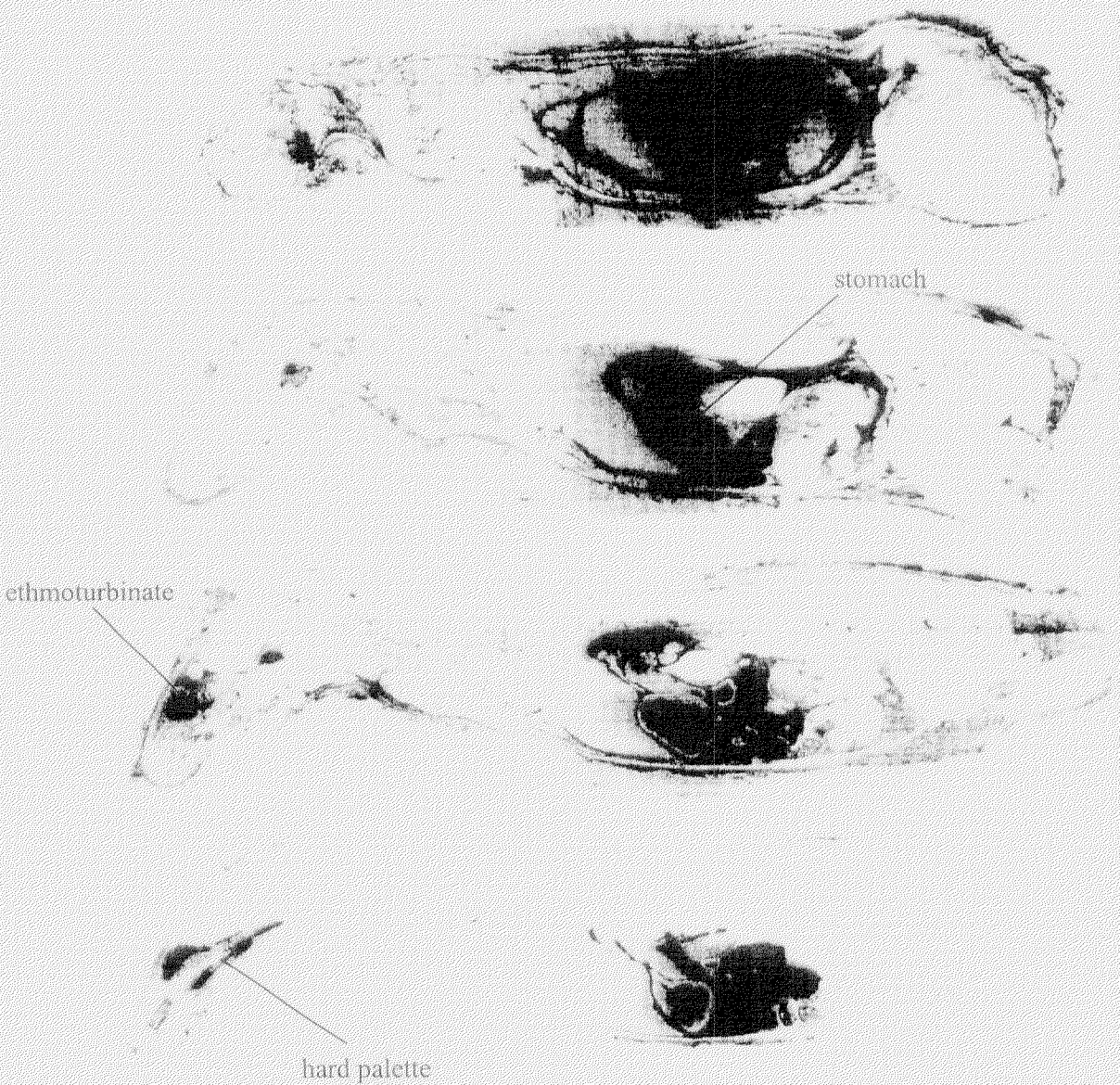
Whole Body Autoradiograph of Female Fischer 344 Rat 1 Hour
Following Administration of a Single Oral Dose of D6 in Corn Oil



D0143 Female 1 hour

Figure 2

Whole Body Autoradiograph of Male Fischer 344 Rat 1 Hour
Following Administration of a Single Oral Dose of D6 in Corn Oil



D0150 Male 1 hour

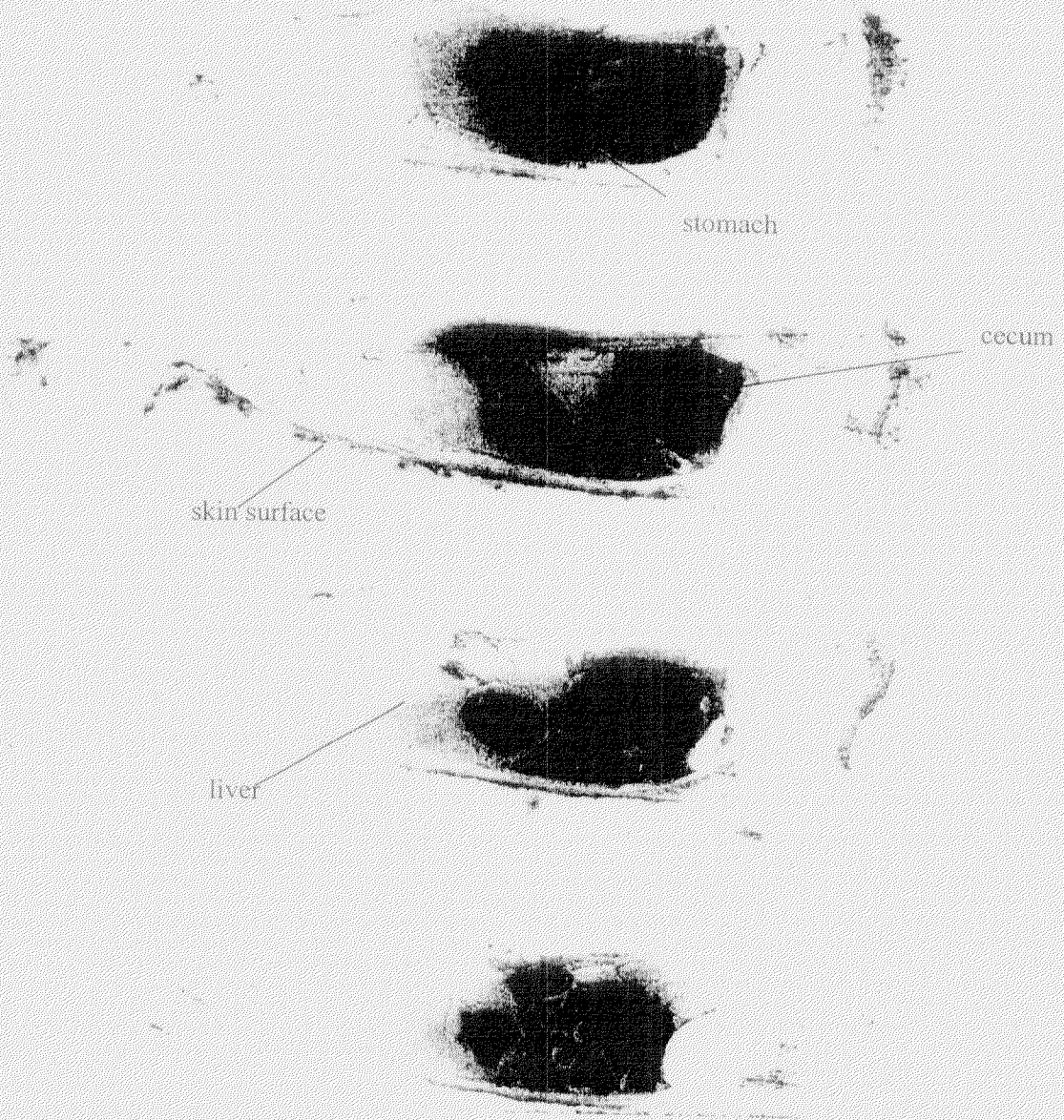
Figure 3

Whole Body Autoradiograph of Female Fischer 344 Rat 4 Hours
Following Administration of a Single Oral Dose of D6 in Corn Oil

D0144 Female 4 hour

Figure 4

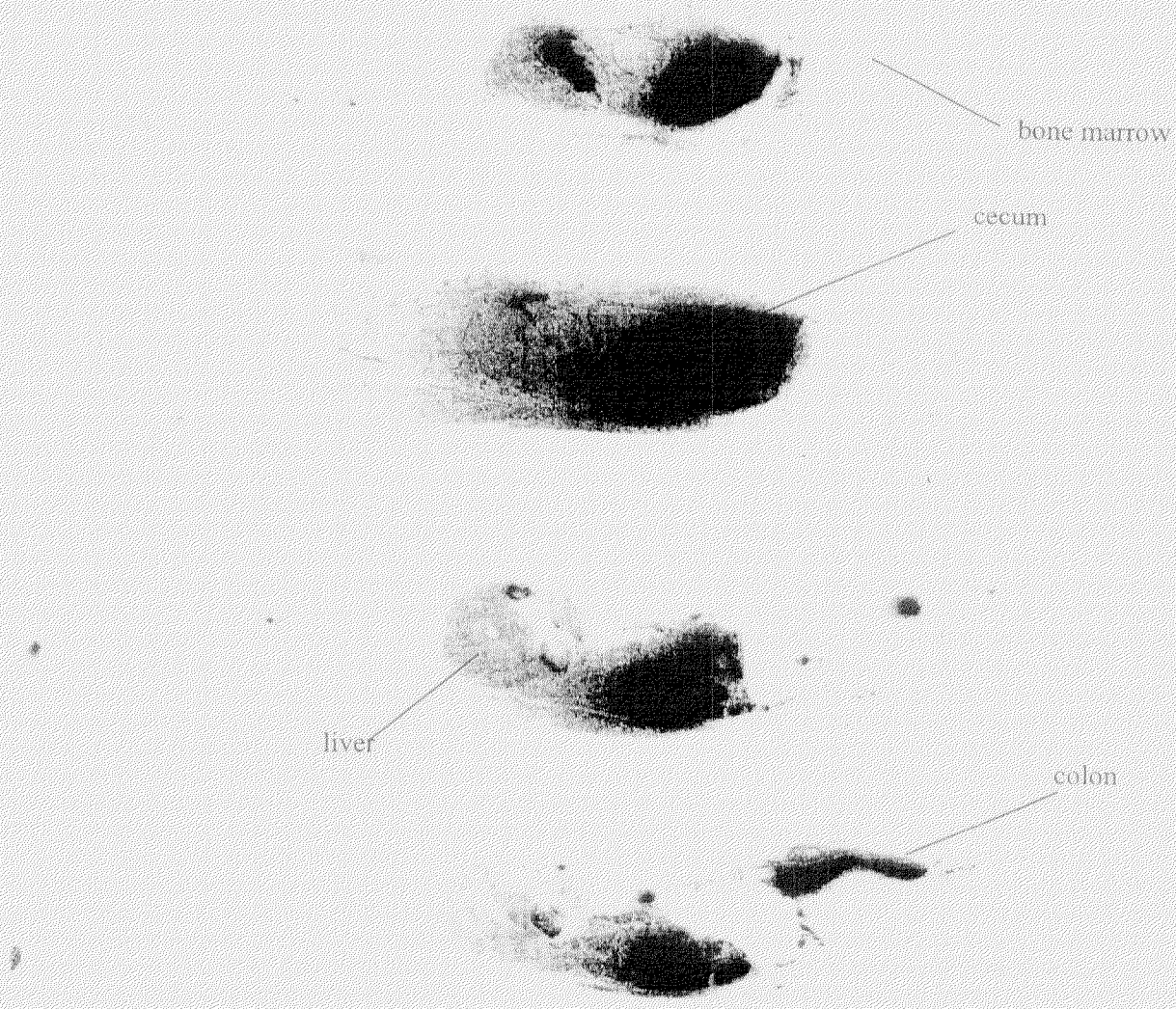
Whole Body Autoradiograph of Male Fischer 344 Rat 4 Hours
Following Administration of a Single Oral Dose of D6 in Corn Oil



D0151 Male 4 hour

Figure 5

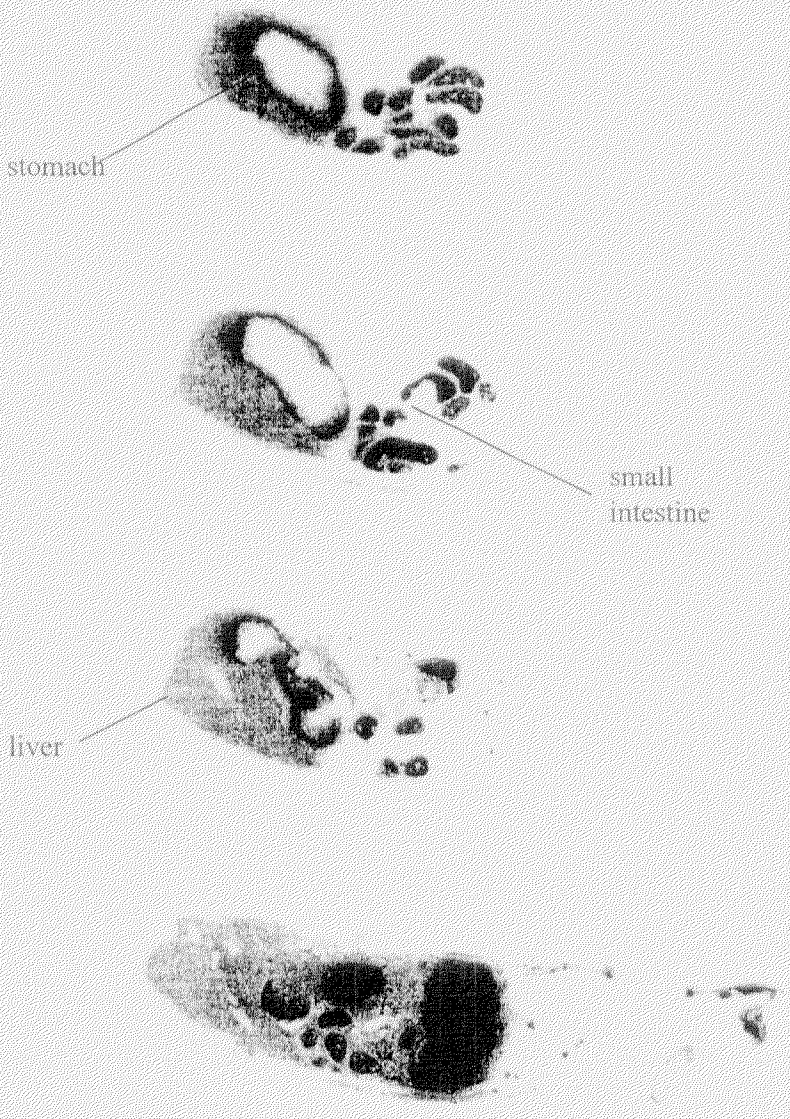
Whole Body Autoradiograph of Female Fischer 344 Rat 12 Hours Following Administration of a Single Oral Dose of D6 in Corn Oil



D0145 Female 12 hour

Figure 6

Whole Body Autoradiograph of Male Fischer 344 Rat 12 Hours Following Administration of a Single Oral Dose of D6 in Corn Oil



D0152 Male 12 hour

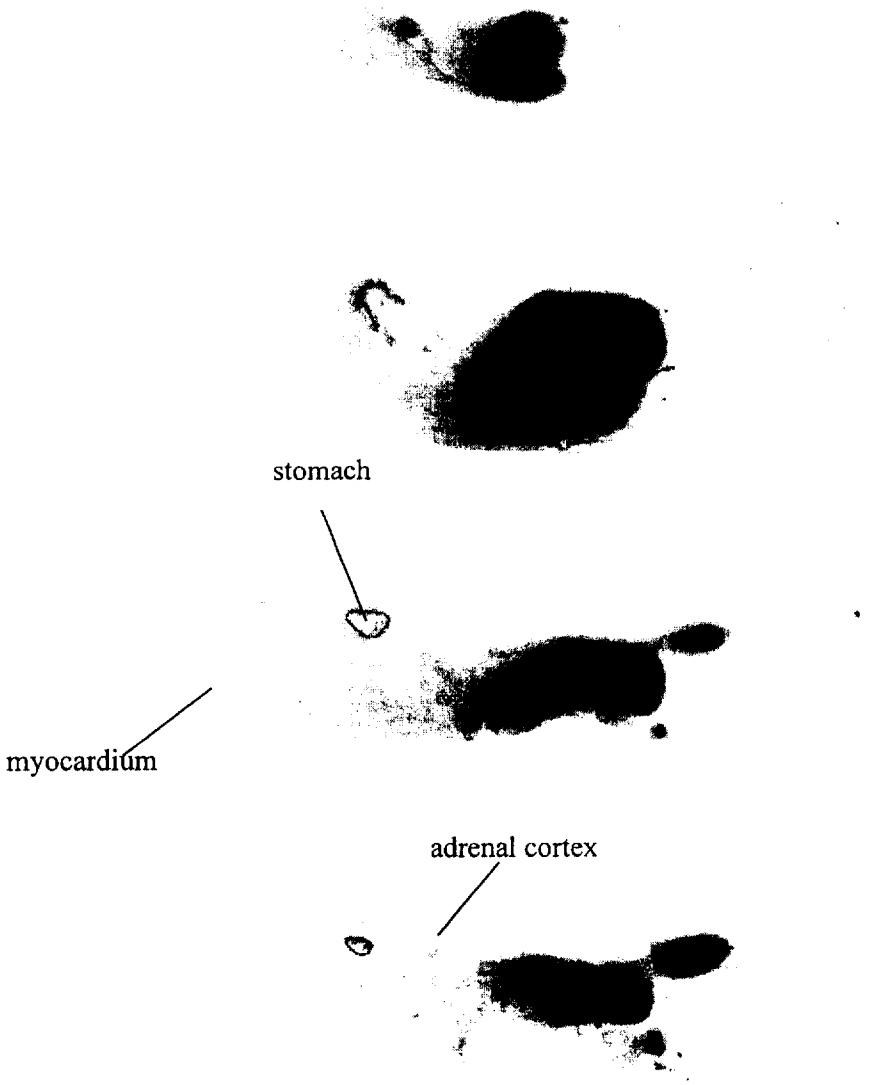
Figure 7

Whole Body Autoradiograph of Female Fischer 344 Rat 24 Hours Following Administration of a Single Oral Dose of D6 in Corn Oil

D0146 Female 24 hour

Figure 8

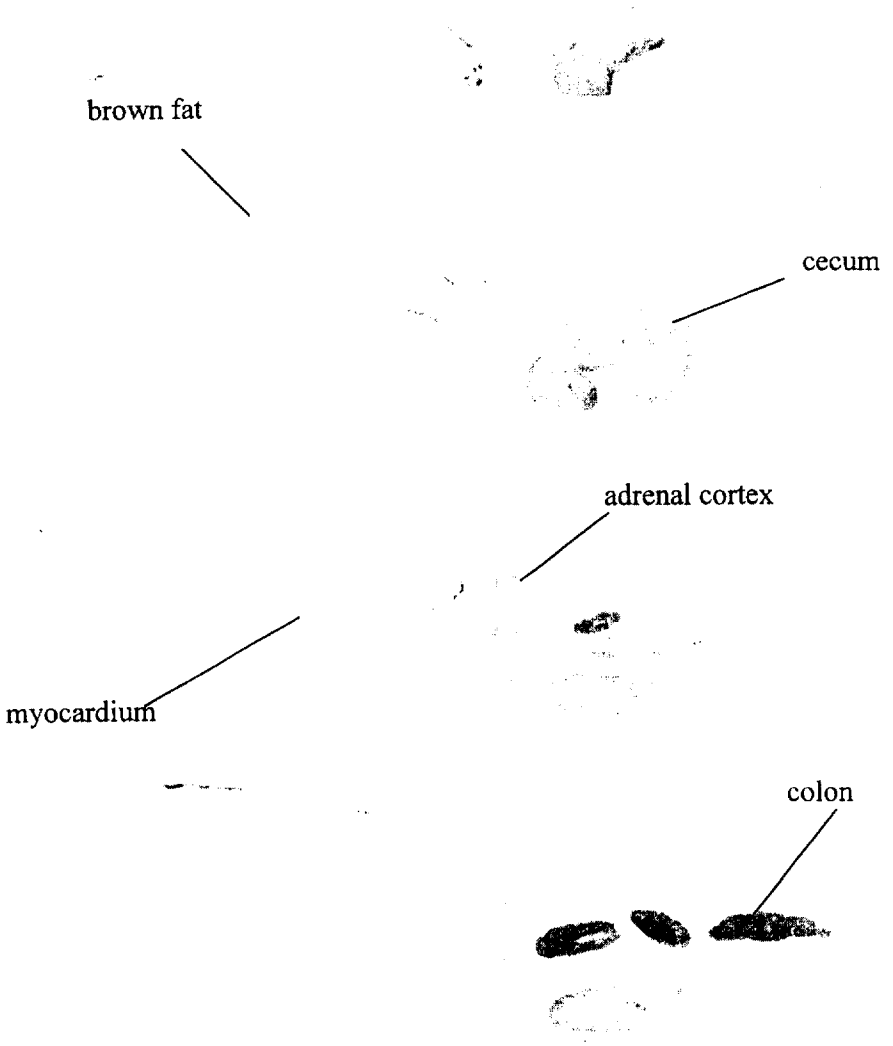
Whole Body Autoradiograph of Male Fischer 344 Rat 24 Hours
Following Administration of a Single Oral Dose of D6 in Corn Oil



D0153 Male 24 hour

Figure 9

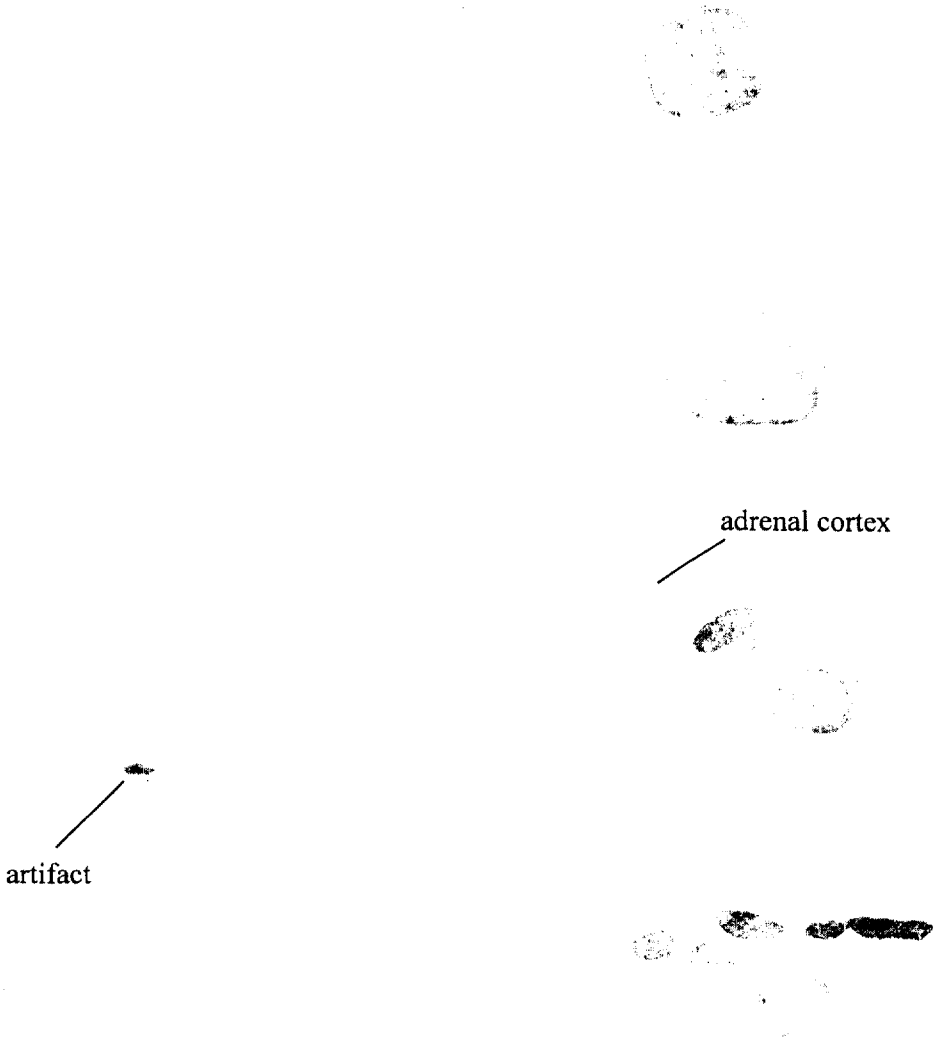
Whole Body Autoradiograph of Female Fischer 344 Rat 48 Hours
Following Administration of a Single Oral Dose of D6 in Corn Oil



D0147 Female 48 hour

Figure 10

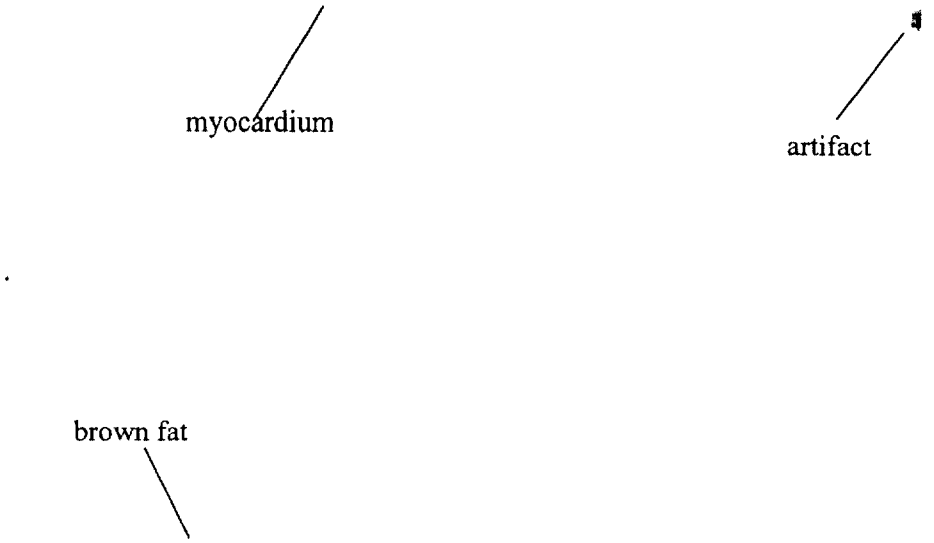
Whole Body Autoradiograph of Male Fischer 344 Rat 48 Hours
Following Administration of a Single Oral Dose of D6 in Corn Oil



D0154 Male 48 hour

Figure 11

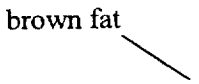
Whole Body Autoradiograph of Female Fischer 344 Rat 96 Hours
Following Administration of a Single Oral Dose of D6 in Corn Oil



D0148 Female 96 hour

Figure 12

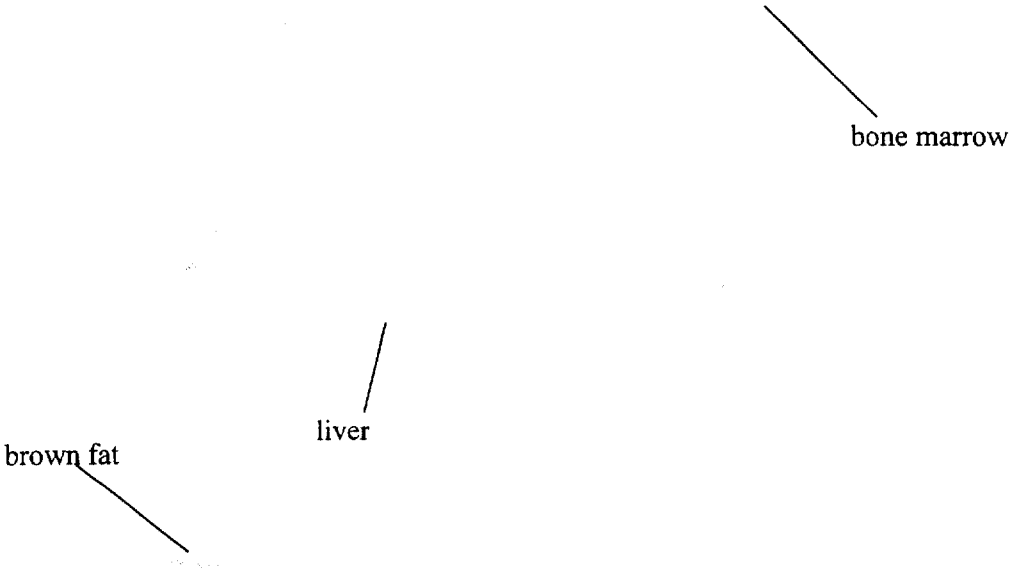
Whole Body Autoradiograph of Male Fischer 344 Rat 96 Hours
Following Administration of a Single Oral Dose of D6in Corn Oil



D0155 Male 96 hour

Figure 13

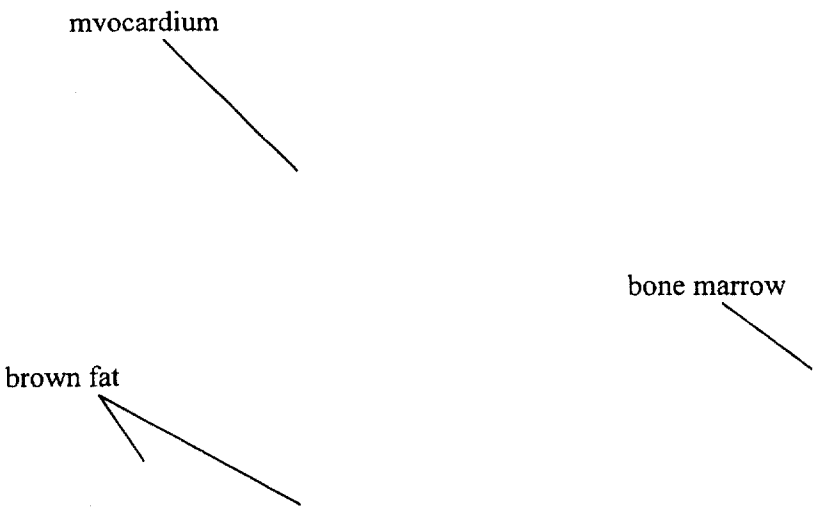
Whole Body Autoradiograph of Female Fischer 344 Rat 168 Hours
Following Administration of a Single Oral Dose of D6 in Corn Oil



D0149 Female 168 hour

Figure 14

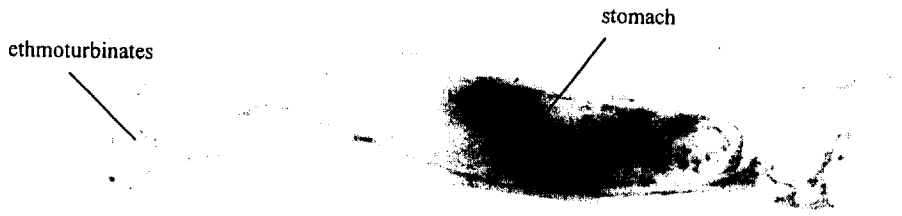
Whole Body Autoradiograph of Male Fischer 344 Rat 168 Hours
Following Administration of a Single Oral Dose of D6 in Corn Oil



D0156 Male 168 hour

Figure 15

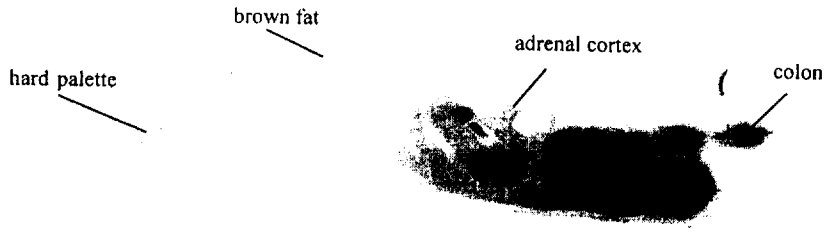
Representative Autoradiographs by Time Point of Female Fischer 344 Rats Following Administration of a Single Oral Dose of D6 in Corn Oil



1 hour

4 hours

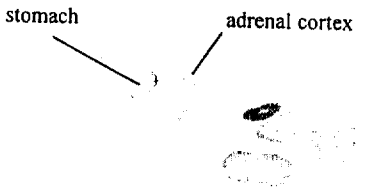
12 hours



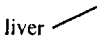
24 hours

Figure 15 (continued)

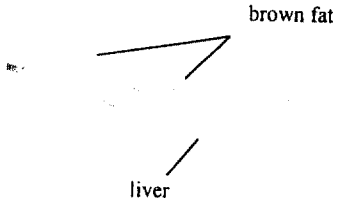
Representative Autoradiographs by Time Point of Female Fischer 344 Rats
Following Administration of a Single Oral Dose of D6 in Corn Oil



48 hours



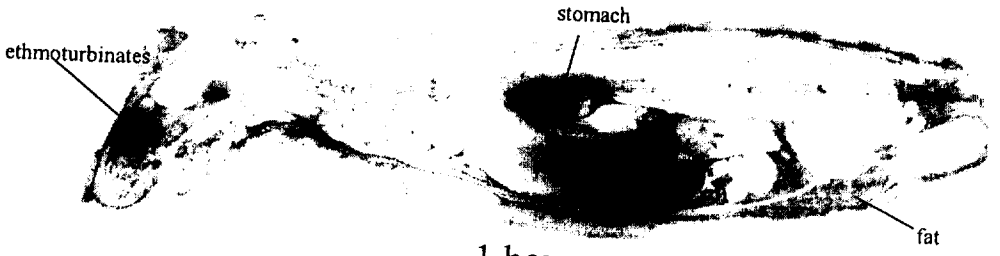
96 hours



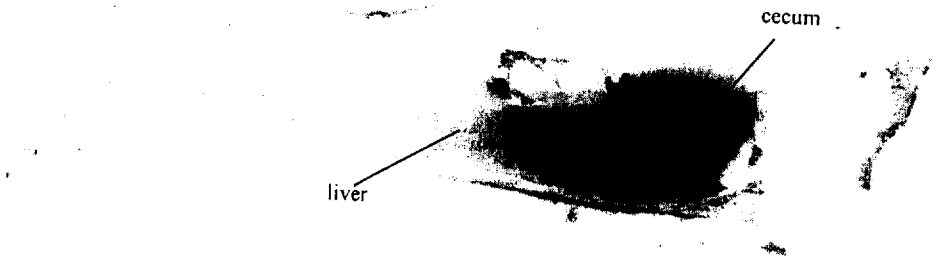
168 hours

Figure 16

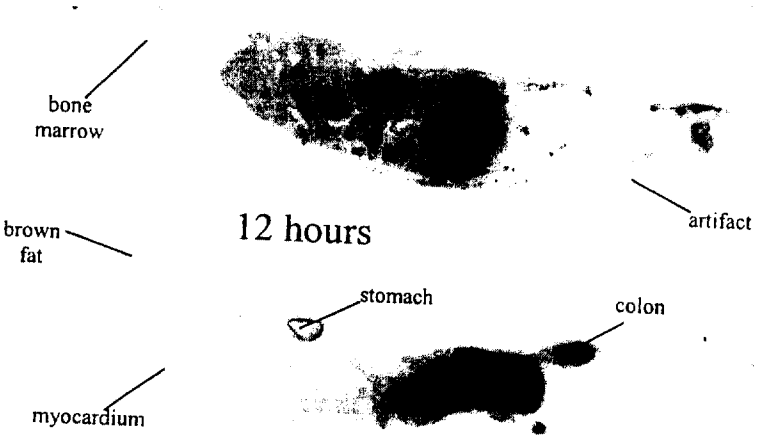
Representative Autoradiographs by Time Point of Male Fischer 344 Rats Following Administration of a Single Oral Dose of D6 in Corn Oil



1 hour



4 hours

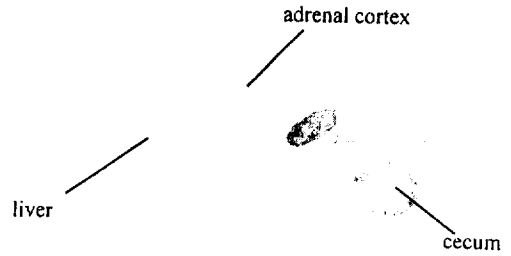


12 hours

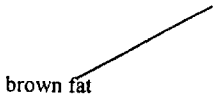
24 hours

Figure 16 (continued)

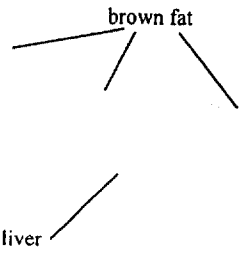
Representative Autoradiographs by Time Point of Male Fischer 344 Rats
Following Administration of a Single Oral Dose of D6 in Corn Oil



48 hours



96 hours



168 hours